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Auditory Masking and the Critical Band in Atlantic Cod (*Gadus morhua*)

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1969

Udo Buerkle

Fisheries Research Board of Canada
Biological Station, St. Andrews, N.B.

Buerkle, Udo. 1969. Auditory masking and the critical band in Atlantic cod (*Gadus morhua*). J. Fish. Res. Bd. Canada 26: 1113–1119.

Auditory masking was determined for cod by using five half-octave bands as masking stimuli and as signals. For each of 15 cod, masking noise was kept constant at 10 db re 1 microbar in one band while thresholds were determined for signals in all five bands. Results indicate masking to be most pronounced when noise and signal coincide in frequency, and to drop off as frequency separation between noise and signal increases. Masking is calculated in terms of threshold in relation to masking noise level, and varies from about 11 db when signal and noise are in the same band to about –19 db when they are in bands furthest removed from each other. Estimates of critical bands are made from the results.

Received December 12, 1968

INTRODUCTION

The natural environment of fishes, and especially that of marine fishes, is known to be noisy. In addition to measures of frequency range and sensitivity, quantitative descriptions of the hearing capacities of fish should, in order to be applicable to natural conditions, include measures of masking and of a critical band. The critical band hypothesis that a tone at threshold in a wide spectrum noise is masked only by the components of the noise near it has long been upheld for human subjects. In fishes, the frequency range and sensitivity of auditory perception have been described many times (Cohen and Winn, 1967; Enger, 1966; Kleerekoper and Chagnon, 1954; Tavolga and Wodinsky, 1963; and others); the effects of masking have not been described until recently.

Two papers report the measurement of the masking effect of noises on pure tones that are within the frequency range of the masking noise. The experiments of Tavolga (1967) describe masked thresholds of the squirrelfish (*Holocentrus rufus*) and of the blue-striped grunt (*Haemulon sciurus*); those of Buerkle (1968) describe masked thresholds of the Atlantic cod (*Gadus morhua*). In the present experiment the masking effect of noises on signals with frequencies differing from those of the masking noise was measured as well as the effect on signals with similar frequencies. Critical bands were estimated from the results.

METHOD

First attempts to use pure tone signals masked by one-half-octave-band noises were abandoned because of difficulties caused by standing waves. Both signals and masking noises were produced in half-octave bands by essentially similar systems (Fig. 1). For each, random white

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noise from a General Radio Random Noise Generator (Model 1390B) was filtered to the desired band by an Allison Lab. Model 2AB band pass filter, and attenuated by a Hewlett Packard Model 350D Attenuator, then amplified by a 50-w RCA Program Amplifier and produced through a University Sound 15-inch speaker in a tuned bass reflex cabinet.

The signal-producing system had a 600-ohm "T" pad, which was used to turn the signal on and off without producing switching transients or impedance effects, between the filter and the attenuator.

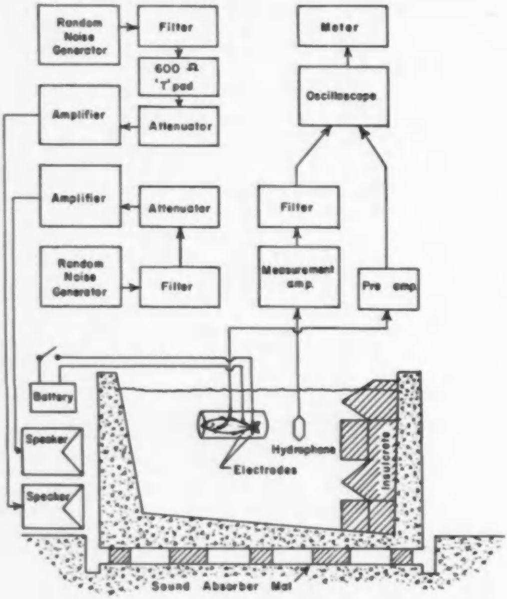
Random Noise Generator
Filter
600 Ω 'T' pad
Attenuator
Amplifier
Amplifier
Attenuator
Random Noise Generator
Filter
Meter
Oscilloscope
Filter
Measurement amp.
Pre amp
Battery
Speaker
Speaker
Electrodes
Hydrophone
Insulcrete
Sound Absorber Mat

FIG. 1. Block diagram of instrumentation and cross section of experimental tank.

The noise system was calibrated so that masking noise could be produced at 10 db re 1 microbar in five half-octave bands. The bands were numbered consecutively and the 3-db down points of bands 1, 2, 3, 4, and 5 were 17.7–25, 35.3–50, 70.7–100, 141–200, and 283–400 Hz, respectively.

The signal system was set up so that signals could be produced in the same five bands but at variable and known intensity levels.

With these systems, any combination of the bands of signal and of masking noise could be produced together.

The noise-measurement system and the experimental tank together with the method of preparing fish for experiments have been described previously (Buerkle, 1967). The procedure of threshold determination was a modification of the procedure previously used. To speed up the process so that a fish could be tested at the five bands in 1 day, the slow decrease (2-db steps) towards threshold with the signal pressure was either speeded up by using 4-db steps, or was eliminated. In the latter case, the fish was trained as in previous experiments to give consistent responses at high levels. It was then given a 15-min rest after which thresholds were determined by successively raising the signal from subthreshold levels until a response was obtained. The critical point in this procedure is to detect the first positive response, and to reinforce it with the conditioning stimulus (6 v d-c) immediately. This procedure worked well with some specimens; other specimens did not respond at all. The threshold levels that were determined by it, however, did not seem to differ from those determined by the other method. On the other hand, the method using 4-db steps to approach thresholds worked well and resulted in consistently successful determinations.

As in previous experiments, care was taken to locate fish in the tank so that relatively uniform sound pressure was presented to them. In this experiment, pressure had to be uniform in the noise band as well as in the signal band. An area large enough to contain the largest fish used was located; average signal pressure and average noise pressure, each calculated from measurements at 10 points in the area, were within ±1 db of the pressure at the point, in the centre of the area, that was used as the calibration point.

Pressure-level calibration of noise and signal bands was checked after each fish but the small variation found was ignored because adjustments were made after each check. A low level of background noise was also present in the tank. It was measured in the same five bands in which signal and noise were produced. It was −17, −17, −23, −31, and −36 db re 1 microbar in bands 1, 2, 3, 4, and 5, respectively.

The fish to be tested was suspended in the area of uniform pressure overnight. Next day the masking noise was turned on about 30 min before training and testing began and was left on throughout the tests. Thresholds were determined for signals in all five bands; the fish was then discarded. With masking noises in all bands except band 1, signal thresholds were determined in bands 1–5 consecutively. With the masking noise in band 1, the order of signal bands tested was 2, 1, 3, 4, and 5. Three fish were tested with each masking noise, but each fish was tested with one noise only.

RESULTS

Thresholds are presented in Table 1. Except in signal band 5, they were masked most pronouncedly when signal and noise were in the same band. With signal and noise in band 1, thresholds of all three fish tested were +21 db. With signal and noise in band 2, they were all 19 db. With signal and noise

TABLE 1. Thresholds (db re 1 microbar) of individual fish for each signal band, with masking noise in five bands.

Masking noise band	Geometric midpoint (Hz)	Fish no.	Signal band				
			1 21 Hz	2 42 Hz	3 84 Hz	4 168 Hz	5 340 Hz
1	21	2	21	7	−1	−13	–
		7	21	9	1	−11	15
		15	21	15	7	−3	25
2	42	1	5	19	9	−3	23
		3	1	19	7	−1	23
		8	5	19	5	1	23
3	84	4	−11	−1	21	5	23
		10	−7	−3	21	5	21
		16	−9	−5	21	3	17
4	168	5	−7	−11	7	21	29
		12	−3	−9	5	21	27
		18	−7	−3	5	19	21
5	340	6	−5	−3	−3	−1	21
		13	−11	−7	−9	−9	19
		19	−9	−11	−1	5	25

in band 3, they were again 21 db, and with signal and noise in band 4, they were 21, 21, and 19 db. The lack of variation among the three fish tested in each situation and the lack of variation among the four situations are remarkable. It suggests that there is virtually no variation in intensity discrimination between different animals and that this capacity is independent of frequency. It is possible, of course, that the lack of variation is due to some unknown characteristic of the sound stimuli used.

In band 5, thresholds were apparently high enough to be uninfluenced by masking of the levels used. Thresholds in bands 1, 2, 3, 4, and 5 determined with noise in band 5 should therefore be unmasked thresholds. However, since there was also the low level of uncontrolled background noise in the experimental tank, these thresholds, although unmasked by the masking stiumlus, are likely masked by this background noise. The conventional measure of masking, which is the difference between masked and unmasked thresholds, can therefore not be calculated.

A measure of the effect of noise on thresholds can be obtained by relating the masked thresholds to the level of the masking noise.

In these experiments the masking level was constant at 10 db re 1 microbar. The average difference in decibels between masked threshold and masking level was calculated at each signal and noise combination (Table 2). These values were plotted on the linear axis of signal frequency and noise frequency by their geometric midpoints (Fig. 2). Points of equal difference were located by interpolation, and joined by smooth curves. The curves are labelled in decibels and indicate the number of decibels a signal at threshold is above or below a masking noise of 10 db re 1 microbar.

TABLE 2. Difference (in decibels) between average thresholds and the masking noise level (10 db re 1 microbar).

Noise band	Geometric midpoint (Hz)	Signal band				
		1 21 Hz	2 42 Hz	3 84 Hz	4 168 Hz	5 340 Hz
1	21	11.0	.3	–7.7	–19.0	10.0
2	42	–6.3	9.0	–3.0	–11.0	13.0
3	84	–19.0	–13.0	11.0	–5.7	10.3
4	168	–15.7	–17.7	–4.3	10.3	15.7
5	340	–18.3	–17.0	–14.3	–11.7	11.7

Previous experiments on cod (Buerkle, 1967) indicated that the decibel difference between threshold and noise is constant for a wide range of noise levels (+4 db to –14 db re 1 microbar). The above relationship can be expected to hold true for a wide range of noise level as well.

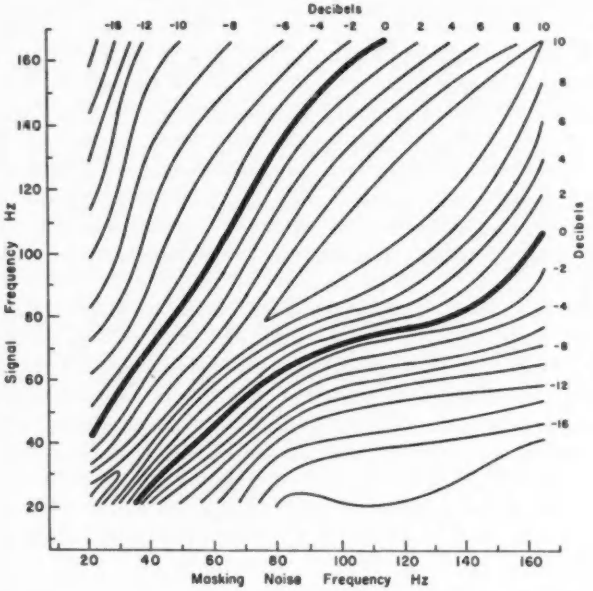

FIG. 2. Contour plot of masking in decibels of signal threshold in relation to masking noise level.

DISCUSSION

Measurements of auditory masking in fish other than cod have been made for only two species. Tavolga (1967) determined thresholds of pure tones masked by bands of noise for the longspine squirrelfish (*Holocentrus rufus*) and the blue-striped grunt (*Haemulon sciurus*). Thresholds of the squirrelfish ranged from 3 to 13 db above noise level in the region of the signal frequency. The bands in which noise was measured are given only for signal frequency 500 Hz, in which case it is specified to have 3-db down points of 380 and 650 Hz. Thresholds of the squirrelfish therefore range from about 27 to 37 db above spectrum level of noise. This, as will be seen, is higher than values for cod at 35.3 and 70.7 Hz.

In the grunt, Tavolga found thresholds at 500 Hz to be equal to noise in the 380- to 650-Hz band. This means that they were about 24 db above spectrum level of noise. This approaches values for cod more closely, but, as with the squirrelfish, direct comparisons cannot be made because of the discrepancy in frequencies.

An estimate of the critical band was made by Tavolga for the grunt. At 500 Hz he estimated it to be 100–150 Hz wide. An estimate of the critical band in cod can be made from the results of this experiment.

Masking represents the inability of the auditory apparatus to separate the tonal stimulation into components and to discriminate between the presence and the absence of one of them (Licklider, 1951). The masking noise in this experiment was a tonal stimulation at 10 db re 1 microbar. As the signal frequency is moved away from noise frequency, thresholds decrease. The frequency separation between signal and noise, at which the threshold is 10 db re 1 microbar, represents a band outside of which signals at 10 db can be detected, but inside of which they cannot be detected. Inside the band then, the auditory apparatus of the fish cannot separate the tonal components. This band is an estimate of critical band, and is represented by the 0-db contours in Fig. 2.

For each fish, signal frequencies were moved about a stationary noise frequency. To keep errors to a minimum, estimates of critical bands should therefore be based on noise frequencies rather than on signal frequencies. Thus, in Fig. 2, the critical band of any frequency on the noise frequency scale is estimated by the frequency separation along the signal frequency scale between the 0-db contours. For example, the critical band for 35 Hz is approximately 21–63 Hz (i.e., 42 Hz), that at 70.7 Hz is approximately 55–117 Hz (i.e., 62 Hz).

An estimate of the validity of these values can be obtained by reference to earlier work on cod. One experiment (Buerkle, 1968) showed pure tone thresholds of cod at 35.3 and 70.7 Hz to be 4.4 and 4.9 db above masking noises produced in octave bands centred at these frequencies. The present experiment indicates that the critical bands at these two frequencies include practically all of the masking noise band to the 3-db down points (i.e., 25–50 for 35.3 Hz and 50–100 for 70.7 Hz). In other words, for pure tones of 35.3 and of 70.7 Hz to be heard in a masking noise, they must be at least 4.4 and 4.9 db above the masking noise in the critical band. The critical bands of 42 and 62 Hz at 35.3 and 70.7 Hz mean that the band levels of the critical bands are 16.2 and 17.9 db respectively above spectrum level. If we add to this the 4.4 and the 4.9 db needed above band level, we get critical ratios of 20.6 and 22.8 db. Pure tones at 35.3 and at 70.7 Hz then must be at least 20.6 and 22.8 db respectively above spectrum level of noise.

Another experiment on cod (Buerkle, 1967) showed thresholds for 35.3 and 70.7 Hz to be 23.9 ± 1.6 and 24.2 ± 1.9 db, respectively, above the upper 95% confidence limit of spectral level noise. At 70.7 Hz this almost coincides with the estimated value of 22.8 db. At 35.3 Hz the measured ratio is somewhat above the estimated minimum of 20.6 db. This, however, may in part be accounted for by the fact that the spectrum of background noise that was used to determine the measured ratio was not flat. It showed a rise at the upper end of the 25–50 Hz band. Correction for this would reduce the measured ratio. In the light of previous work, the estimates of critical band in cod seem valid.

ACKNOWLEDGMENTS

I am grateful to Mr S. M. Polar for his help with the design and setting up of the electronics and to Dr J. M. Anderson for critical reading of the manuscript.

REFERENCES

BUERKLE, UDO. 1967. An audiogram of the Atlantic cod *Gadus morhua* L. J. Fish. Res. Bd. Canada 24: 2309–2319.

1968. Relation of pure tone thresholds to background noise level in the Atlantic cod (*Gadus morhua*). J. Fish. Res. Bd. Canada 25: 1155–1160.

COHEN, M. J., AND H. E. WINN. 1967. Electrophysiological observations on hearing and sound production in the fish, *Porichthys notatus*. J. Exptl. Zool. 165: 355–369.

ENGER, PER S. 1966. Acoustic threshold in goldfish and its relation to the sound source distance. Comp. Biochem. Physiol. 18: 859–868.

KLEEREKOPER, H., AND E. C. CHAGNON. 1954. Hearing in fish, with special reference to *Semotilus atromaculatus atromaculatus* (Mitchill). J. Fish. Res. Bd. Canada 11: 130–152.

LICKLIDER, J. C. R. 1951. Basic correlates of the auditory stimulus, p. 985–1039. *In* S. S. Stevens [ed.] Handbook of experimental psychology. John Wiley & Sons, Inc., New York.

TAVOLGA, W. N. 1967. Masked auditory thresholds in teleost fish, p. 233–243. *In* W. N. Tavolga [ed.] Marine bio-acoustics, Vol. 2. Pergamon Press, New York.

TAVOLGA, W. N., AND J. WODINSKY. 1963. Auditory capacities in fishes. Pure tone thresholds in nine species of marine teleosts. Bull. Am. Museum Nat. Hist. 126: 179–239.

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Canadian Atlantic Oyster Drills (*Urosalpinx*) — Distribution and Industrial Importance

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MEDCOF, J. C., AND M. L. H. THOMAS. 1969. Canadian Atlantic oyster drills (*Urosalpinx*) — distribution and industrial importance. J. Fish. Res. Bd. Canada 26: 1121–1131.

Distribution of drills (*Urosalpinx cinerea* Say) was worked out from critical examination of museum holdings, from assessment of published and several unpublished occurrence records, and from field collection in new areas. Drills occur in warm inlets on the Nova Scotia coast of the Bay of Fundy, possibly in one inlet of the outer Atlantic coast of Nova Scotia, and in several inlets of the southwestern Gulf of St. Lawrence. Only in the last were oysters found with the drills and there is only one record of serious damage to young oysters. Distribution patterns suggest that drills reached the Gulf of St. Lawrence by spreading eastward from Cape Cod, through the Bay of Fundy, then through a channel that is believed to have connected the Bay and the Gulf. There is no evidence of long-term restriction of distributional range since that pioneering period, but local distribution seems to have contracted so that now drills occupy only the warmest parts of their range. In the last 30 years, drill abundance in some important oyster-producing sections of the Gulf of St. Lawrence has decreased, perhaps because of low sea temperatures. Old colonies persist but do little damage in oyster areas. No new colonies seem to have sprung up. In our area the species seems to be bordering on extinction. Drills are not a threat to the Canadian east-coast oyster industry.

Received November 18, 1968

INTRODUCTION

ON THE EAST COAST of the United States, oyster drills are widespread, abundant, and destructive oyster pests (Galtsoff, 1964). They also occur in eastern Canada (Bousfield, 1960) and are reported to have destroyed as much as 30% of the annual set of young oysters (Adams, 1947, MS, 1952) in one oyster area in 1939.

Drills migrate slowly (Ganong, 1889; Carriker, 1955) and have no free-swimming larval stage. Consequently their natural spread is likely to be slow. However, oyster culturists have inadvertently introduced this species to the Pacific coast of North America and to the Atlantic coast of Europe where it has become a pest (Galtsoff, 1964).

Canadian east-coast oyster farmers regularly transplant oysters in bulk from one area to another and from 1957 to 1962 the Department of Fisheries made massive transplants in the Gulf of St. Lawrence to rehabilitate oyster

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[1] Present address (M.L.H.T.): Fisheries Research Board of Canada, Oyster Unit, Ellerslie, P.E.I.

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stocks (Drinnan and England, 1965). The Ministry of Fisheries has long feared that these transfers would spread drills to new areas where they might establish destructive new colonies. For this reason it has frequently urged industry to exercise precautions to offset this risk (Adams, 1947; Medcof, 1961).

The purpose of this study was threefold: (1) to obtain comprehensive, reliable information on drill distribution by compiling and assessing earlier records, by studying museum collections, and by searching for drills in areas where information was lacking; (2) to explain present drill-distribution patterns in terms of factors that seem to have controlled drill abundance and drill distribution both recently and in early post-Pleistocene times; and (3) to assess chances that drills will increase in abundance and spread either naturally or artificially in our industrially important oyster areas.

All available collections referred to in this study have been examined by one or both authors and identified with assistance from Dr Arthur H. Clarke of the National Museum of Natural Sciences (NMNS), Ottawa, Ont.

OCCURRENCE RECORDS

Occurrence records are summarized in Fig. 1 and in Table 1, which includes our assessment of them. Some of these records are discussed below.

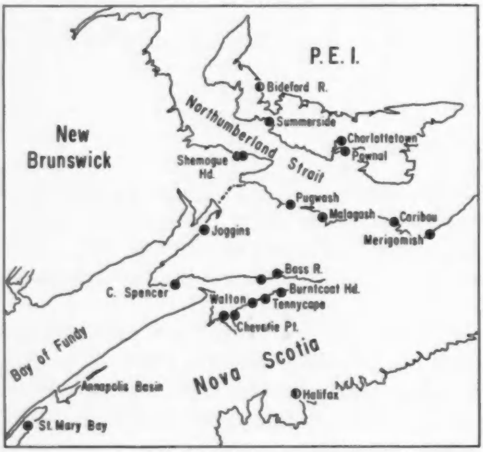
P. E. I.
Bideford R.
Northumberland Strait
Summerside
Charlottetown
Pownal
New Brunswick
Shemogue Hd.
Pugwash
Malagash
Caribou
Merigomish
Joggins
Bass R.
Burntcoat Hd.
C. Spencer
Walton
Tennycape
Chevarie Pt.
Bay of Fundy
Nova Scotia
Annapolis Basin
Halifax
St. Mary Bay

FIG. 1. Sites of collections of oyster drills, or their shells, in Canadian Maritime Provinces. Solid circles indicate valid distribution records; half-solid circles indicate collections of shells of likely advectitious origin (Table 1).

EARLY RECORDS

Sir William Dawson's (1871) is the oldest published record (Table 1). The only extant specimen collected by him is in the Carpenter collection in the Redpath Museum (RM). The locality shown on the label is simply, "Prince Edward Island."

TABLE 1. Summarized occurrence records of oyster drills on Canadian Atlantic coast and our appraisal of them. P.E.I. = Prince Edward Island; N.S. = Nova Scotia; N.B. = New Brunswick; RM = Redpath Museum, McGill University, Montreal; NMNS = National Museum of Natural Sciences, Ottawa; ROM = Royal Ontario Museum, Toronto; FRB = Fisheries Research Board of Canada, Oyster Unit, Ellerslie, P.E.I.; Mt.A. = Mount Allison University, Department of Biology, Sackville, N.B.; OK = acceptable record.

Year	Collector or reporter	Locality of collection	Repository of collection	Our assessment of record
		Old records		
1871	Dawson	Prince Edward Island	RM	OK
1865–76(?)	Willis	Halifax, N.S.	RM	Not critically valid
1873	Verrill	Gulf of St. Lawrence; southern part (apparently no new collection)	None	OK
1885	Bain	Prince Edward Island	NMNS(?) (see text)	OK
1906	Chadwick	Northumberland Strait, P.E.I.; in deep water	None	Invalid
1907	Ives	Northumberland Strait, P.E.I.; in deep water	None	Invalid
1916	Kindle	Minas Basin, N.S.; at mouth of Avon River opposite Cheverie Point	None	OK
		Recent records		
Bay of Fundy				
1920–22	Leim	Minas Basin, N.S.; at Bass River and Tenny Cape	ROM	"
1944	Medcof	Minas Basin, N.S.; common at Bass River feeding on barnacles	Identifications checked by Leim; no specimens retained	"
1958	Bousfield and Leim, 1960; Bousfield, personal communication, 1967	Minas Basin, N.S.; at Walton, Burntcoat Head, Spencer Point, and Economy Point	NMNS	"
1963	Bousfield, personal communication, 1967	St. Mary Bay, N.S.; at Brighton	NMNS	"
1968	Arnold, personal communication, 1968	Cumberland Basin, at Joggins, N.S.; one large recently dead shell containing hermit crab	Mt.A.	Reliable shell record
Gulf of St. Lawrence				
1939	Adams	Tatamagouche Bay, N.S.; abundant on oyster bars in Malagash Basin; Caribou Harbour, N.S.; abundant on oyster lease	FRB and NMNS	OK

(Continued)

TABLE 1. Summarized occurrence records of oyster drills on Canadian Atlantic coast and our appraisal of them. P.E.I. = Prince Edward Island; N.S. = Nova Scotia; N.B. = New Brunswick; RM = Redpath Museum, McGill University, Montreal; NMNS = National Museum of Natural Sciences, Ottawa; ROM = Royal Ontario Museum, Toronto; FRB = Fisheries Research Board of Canada, Oyster Unit, Ellerslie, P.E.I.; Mt.A. = Mount Allison University, Department of Biology, Sackville, N.B.; OK = acceptable record. — (*Concluded*)

Year	Collector or reporter	Locality of collection	Repository of collection	Our assessment of record
1939–40	Ingalls	Caribou Harbour, N.S.; plentiful at MacKenzie and Doctor islands; between Spring and Oak points; mouths of Caribou and Little Caribou rivers: Merigomish Harbour, N.S.; present but not common at Pig Island; in Pine Tree Gut: Pugwash River, N.S.; present but not common at its confluence with Dougherty's Creek; on the shore between confluences of Pugwash River with Dougherty's Creek and Canfield Creek	None	OK
1939	Needler	Pownal Bay, P.E.I.; a few egg cases	None	"
1943	Medcof	Bedeque Bay, P.E.I.; egg cases with embryos and drilled oysters in Summerside Harbour off Wilmot Point	NMNS and FRB	"
1944	Adams, personal communication, 1944	Bedeque Bay, P.E.I., several adult drills from Wilmot Point	None	"
1955	Carriker	Wide distribution, e.g., Magdalen Islands and Caraquet Bay	None	Invalid
1964	Bourne and Rowell, MS, 1965	Northumberland Strait; abundant in one dredge haul off Murray Harbour, depth 35 m (incidental record)	None	Invalid
1966	Olde (Nancy)	Malpeque Bay, P.E.I.; one juvenile "dead" shell on beach at Bideford where Summerside Harbour oysters are regularly relaid	FRB	Identification OK: specimen probably advectitious
1967	Thomas	Bedeque Bay, P.E.I.; off Hurd Point in Summerside Harbour, one living adult drill	FRB	OK
1967	Thomas	Tatamagouche Bay, N.S.; a few drills on Malagash oyster bars below low-water mark	FRB	OK
1968	Arnold, personal communication	Shemogue Head, N.B.; one eroded dead shell	Mt.A.	Arnold and Thomas collections taken together: valid shell records but not sure evidence of living colony
1968	Thomas	0.4 km east of Shemogue Head, N.B.; two eroded dead shells	NMNS	

The Carpenter collection also includes a drill, evidently old, but with a label that is undated and simply lists the collector as "Willis," and the locality as "Halifax." Willis was almost certainly John Robert Willis 1825–1876, "the first Nova Scotian conchologist" (Ganong, 1890a), who lived in the Halifax area and did much of his collecting there. He may have been the first to discover drills in Canada, although Dawson (1871) is and probably will continue to be considered the first. Most biologists question the Willis record because of its sketchy data and possible advectitious[2] origin. The best we can say for the record is that it is not critically valid (Table 1).

Verrill (1873) published the second statement. He seems to have been compiling published information, although he quotes no source. He added nothing new.

Bain's (1885) is the third published record (Table 1) and, like Dawson, he listed only "Prince Edward Island" as the locality. We found no earlier Bain record, although Ganong (1887, 1889, 1890b, c), Whiteaves (1901), and Ives (1907) all quote Bain "(1875)" as having found drills on the south shore of Prince Edward Island. A specimen at the NMNS (Cat. No. 36990) bears this simple inscription, "Charlottetown Harbour, P.E.I." It may have been collected by Bain because Whiteaves (1901), who studied this collection, refers Bain's collection to Charlottetown Harbour.

Chadwick (1906) and Ives (1907) both reported on Ives' collection of unusually large drills from deep water in Northumberland Strait. The Strait is an unlikely habitat for drills, which are a warmwater species (Ganong, 1890b). From this and from the great size of the specimens, we suspect that the "drills" were in fact juvenile rough whelks (*Buccinum undatum* L.). This suspicion is strengthened by the facts that (1) Ives did not include rough whelks in listing his collections although they are abundant in Northumberland Strait; and (2) drills and juvenile rough whelks are frequently confused.

The Ives collection has disappeared and there is no way of checking the Ives and Chadwick identifications. Under these circumstances we reject their records as invalid (Table 1).

We found none of Kindle's (1916) Minas Basin collections in Canadian museums, and correspondence indicates none in United States museums. However, Kindle's reputation for careful work and subsequent collections of drills from other parts of the Basin justify appraisal of his record as acceptable (Table 1).

Recent Records

BAY OF FUNDY

Since 1916, drills have been collected from the Nova Scotia coast of the Bay of Fundy, and many adequately labelled specimens have been deposited in museums. We assessed all these recent records as acceptable (Table 1) except that we have been unable to verify a Bousfield–Leim (1960) beach-shell record for station C18 near Parrsboro, N.S.

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[2] "*Advectitious*" means "brought to a place from a distance; foreign; brought from another place" according to *The Century Dictionary, an encyclopedic lexicon of the English language.*

Dr David Arnold's 1968 record for Joggins is interesting. He looked for it there at our request. There may be other colonies in Cumberland Basin.

GULF OF ST. LAWRENCE

Many new records appeared after 1939 when Adams (1947, MS, 1952) found a dense and destructive colony of drills on oyster bars in Malagash Basin in Tatamagouche Bay, N.S. The following comments will help explain our assessments (Table 1) of some of the new records.

Dr Ingalls' collections (Ingalls and Needler, MS, 1939, MS, 1940) have not been preserved but we feel confident in accepting his identifications and records (Table 1) because Adams and Ingalls worked together at Malagash in 1939 where the species was then abundant and well known.

Coauthor Thomas searched for drills on the Malagash bars at low tide in 1963, 1965, and 1967 but found neither drills nor drilled spat. However, in a 1967 scuba survey he did find a few drills below the low-water mark (Table 1), but no drilled oyster spat.

Few drills have been collected recently on Prince Edward Island. In 1939, Needler (who supervised Adams' work and was well acquainted with drills) found egg cases in Pownal Bay near Charlottetown (Adams, MS, 1952). In 1943, Kerswill searched this area at low tide but found no drills or egg cases (Medcof and Morrison, MS, 1943).

Medcof's 1943 record (Medcof and Morrison, MS, 1943) was confirmed by Adams' 1944 and Thomas's 1967 collections (Table 1) indicating that drills are present but not common in Summerside Harbour, an inlet from Bedeque Bay, P.E.I.

According to Carriker (1955), Gulf of St. Lawrence drills range much further north and occur on the Magdalen Islands, Que., and along the northeastern coast of New Brunswick to Caraquet Bay. He also lists them from Sable Island (in the open Atlantic, 90 miles from shore) and from Passamaquoddy Bay, N.B. Collection records support none of these statements and we have been obliged to asses them as invalid (Table 1). Carriker seems to have been misled by Whiteaves' (1901, p. 7) ambiguous statements.

Bourne and Rowell (MS, 1965) reported drills at a depth of 35 m in Northumberland Strait off Murray Harbour, P.E.I. This record is suspect for the same reasons as Chadwick's and Ives'. Rowell states (personal communication, 1968) that this identification was uncritical. We have assessed the record as invalid (Table 1).

The significance of Miss Nancy Olde's 1966 finding of a "dead" shell at Bideford beach on Malpeque Bay (Table 1) is questionable. This is an area where Summerside Harbour oysters are regularly relaid. Coauthor Thomas has made dredge and scuba surveys of Malpeque Bay annually from 1960 to 1967 without finding drills, and Stafford (1912), Robertson (1916), and Medcof and Morrison (MS, 1943) found none in their dredging surveys. From this we conclude that there is no drill colony in Malpeque Bay and that Olde's specimen was advectitious. It could have been transferred from Summerside Harbour with oyster transplants as indicated in the table.

The 1968 discoveries of shells of drills in the Shemogue, N.B., area, first by Arnold, then by Thomas, are interesting. The two collection sites are far enough apart to remove any suspicion that the specimens were advectitious. However, coauthor Medcof has fishermen's reports of ancient oyster shells being washed ashore on Shemogue Head during severe storms. The same thing happens on the Magdalen Islands and these latter have been carbon-dated as 2175 ± 70 BP (before present) (Medcof et al., 1965). The Shemogue drill shells may also be ancient. So, although the collections constitute a valid shell record, they are not sure evidence of a living colony in the area (Table 1).

DISTRIBUTION PATTERNS

The single early Halifax Harbour record by Willis (Fig. 1) might suggest that there was a colony of drills there about 100 years ago. Since then, however, there have been no confirmatory records from anywhere on the outer coast of Nova Scotia. Furthermore, Bousfield (personal communication) states that he has searched Halifax Harbour and Bedford Basin and failed to find a habitat or a faunal assemblage into which drills might fit. In other words, there is no good evidence of drills on the outer coast.

Recent collections show that there are living colonies in only two regions: (1) in warm bays on the Nova Scotia coast of the Bay of Fundy; and (2) in the southern Gulf of St. Lawrence in warm bays on Northumberland Strait coasts of Prince Edward Island, Nova Scotia, and possibly New Brunswick.

Ganong (1890c) believed that drills must occur on the New Brunswick coast of the Gulf of St. Lawrence, even though Winkley (1888) had failed to find evidence of them at Cocagne. Bousfield (personal communication) also failed to find drills in 1960 during extensive dredging (depths 2–3 m) in Shediac Bay to the west of Indian Island. However, the Arnold and Thomas records do partially vindicate Ganong's faith. Full vindication awaits the discovery of living drills at Shemogue or elsewhere on the New Brunswick coast of the Gulf.

Willis was the first to realize that there are isolated communities of southern invertebrates such as Atlantic oysters (*Crassostrea virginica* Gmelin) and bay quahaugs (*Mercenaria mercenaria* L.) in those inlets of our Atlantic coast that are warmest in summer (Ganong, 1890a, b). South of Cape Cod, these same communities are widespread and include drills, and it is generally agreed that our warmwater species must have come to us from the southwest; but how and when?

EXPLANATION OF PATTERNS

Bousfield (1962, 1967) has tried to explain the presence of warmwater species on our coast and speculates that these communities spread northeastward along the coast and eventually into the Gulf of St. Lawrence commencing

6,000–10,000 years ago during the warm postglacial "hypsithermal" (Deevey and Flint, 1957) period. Sea levels were low then but rising, and our continental shelf waters formed a vast island-studded shallows in which oysters and some other warmwater species achieved wide distribution (Medcof et al., 1965; Clarke et al., 1967). Apparently they colonized the shelf from areas south of Cape Cod (Merrill et al., 1965) northward to Nova Scotia and beyond.

Since then, sea levels have continued to rise, sea temperatures have fallen and distribution of many of these species seems to have contracted so much that they now occur only as the isolated communities that Willis recognized. Today's distribution of drills illustrates this. Moore (MS, 1966) reported drills in Great Bay, New Hampshire, not far from Cape Cod (specimens at NMNS supplied by Bruce Smith). Carriker (1955) describes their occurrence in western Maine (Casco Bay), and Bousfield (personal communication) found two drill colonies on the central Maine coast in 1963. These were in the Damariscotta River estuary where drills have a long history (Carriker, 1955) and in the Medomat River (Greenland Cove, 43°59′N, 69°25′W), which seems to be the first record (specimens at NMNS).

The New Hampshire and Maine relict colonies form a series that extends into our region (Fig. 1).

Bousfield (1967) postulates that the northeastward-spreading warmwater species, which included drills, could have reached the Gulf of St. Lawrence between 10,000 and 6,000 years ago by one or both of two routes: (1) an outside route, along the outer Nova Scotia coast and thence through Cabot Strait or perhaps the Gut of Canso; (2) an inside route, through the Bay of Fundy, and then through a channel that is thought to have connected the Bay of Fundy with the Gulf of St. Lawrence in hypsithermal times (Department of Energy, Mines and Resources, 1968). The position of the hypothetical channel is believed to have been about the same as that of the interprovincial boundary (Nova Scotia–New Brunswick) shown as a broken line in Fig. 1.

If Willis's incompletely documented record had been valid it might have suggested that drills spread along the outside route as far as Halifax Harbour (Fig. 1). Actually there is no good evidence that they spread along this route at all, although diligent collecting may produce new evidence and new interpretations. Available evidence suggests that drills spread along the inside route and reached the Gulf only by the inside (channel) route. They seem to have then spread in Northumberland Strait from the northern end of the channel. In other words, present knowledge of drill distribution supports the channel theory of dispersal for this species.

If the channel theory be valid, Fig. 1 suggests that the pioneering drills spread southeastward in the Strait at least as far as Merigomish Harbour where the Strait widens abruptly. Ingalls found them there (Table 1), and Smith (1929) and Erskine (personal communication) both found dead shells in Indian middens on the shore (specimens at NMNS). Erskine tentatively attribute the midden shells to the period 100 BC to 1200 AD. On the Prince Edward

Island side, the immigrant drills apparently spread southeastward at least as far as Pownal.

On the same theoretical grounds, Fig. 1 suggests that drills spread northwestward from the northern end of the channel at least as far as Shemogue Head on the New Brunswick side of Northumberland Strait and as far as Summerside Harbour on the Prince Edward Island side. Apparently Summerside Harbour shelters the most northern living colony of drills in North America.

The known spread northwesterly and southeasterly from the northern end of the channel is not great (Fig. 1). This suggests either that oceanographic conditions at the time of arrival did not favour wide dispersal or that temperatures decreased from high hypsithermal values soon after arrival. It may be that the geographic range is about the same today as it was then but is more spotty.

PRESENT RISKS OF SPREAD

None of the newly discovered colonies of drills are in areas where Malagash and Summerside oysters have been planted recently, although it is almost certain that these transplants included drills or their egg cases or both. On the contrary, the newly discovered colonies are in places where we expected they would be on the basis of the inside-route theory of spread from the Cape Cod region. Indeed, the search at Joggins, N.S., and Shemogue, N.B., by Arnold and Thomas (Table 1) was made because we were convinced from our knowledge of the habitat and from our faith in the inside-route theory, that they *should* have been there since hypsithermal times.

All this indicates that drills are still not spreading in the Gulf of St. Lawrence, even with man's assistance; they are not pioneering as they were 10,000 years ago. In our areas they are a species that seems to be barely maintaining itself at the northern limits of its range. They may even be on the road to extinction.

We are now in a period of low water temperatures (Lauzier and Marcotte, 1967). This would help to explain why drill abundance is low in areas recently explored and why it seems to have decreased at Malagash in the last 25–30 years. It also suggests that conditions for spread will continue to be unfavourable for some years because return to above-average temperatures is likely to be a long, slow process.

INDUSTRIAL IMPORTANCE

This study indicates that, on our coast, drills are now common only in parts of the Bay of Fundy where there are no oysters and where, consequently, drills have no industrial importance. Our oyster industry is concentrated in the southern Gulf of St. Lawrence. Drills there seem to have survived for several thousands of years but occur only in a few of the oyster areas. In most of them they have always been scarce, so far as we know. In one of the very few areas where they were abundant and destructive 30 years ago (Malagash) they are now scarce, possibly because of lowered water temperatures.

A general statement would be that they are scarce in the Gulf of St. Lawrence, that some colonies are verging on extinction, and that there is no reason to expect remarkable abundance increases.

Furthermore, there is no evidence that their range is extending naturally or being extended artificially, in spite of transfers of oysters from areas where there are drill colonies.

We conclude that drills are not a threat to the Canadian Atlantic oyster industry and that they will not be a threat in the near future.

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REFERENCES

Adams, J. R. 1947. The oyster drill in Canada. Fish. Res. Bd. Canada, Atlantic Progr. Rept. 37: 14–18.
MS, 1952. Oyster investigations at Malagash, N.S., 1939–1942. Fish. Res. Bd. Canada, MS Rept. 472. 63 p.

Bain, F. 1885. The shells of Prince Edward Island. Canadian Sci. Monthly (Publ. Acadian Sci. Club) 3: 33–35.

Bourne, N., and T. W. Rowell. MS, 1965. Gulf of St. Lawrence scallop survey — 1964. Fish. Res. Bd. Canada, MS Rept. (Biol). 809. 33 p.

Bousfield, E. L. 1960. Canadian Atlantic sea shells. Natl. Museum Canada, Ottawa, Ont. 72 p.
1962. Studies of littoral marine arthropods from the Bay of Fundy region. Natl. Museum Canada Bull. 183: 42–62.
1967. Postglacial dispersal patterns of littoral marine molluscs and crustaceans in eastern Canada. (Abstr.) Am. Malacological Union Ann. Rept. 1967: 42–44. (Abstr. only.)

Bousfield, E. L., and A. H. Leim. 1960. The fauna of Minas Basin and Minas Channel. Contrib. Zool. Natl. Museum Canada Bull. 166. 30 p.

Carriker, M. R. 1955. Critical review of biology and control of oyster drills *Urosalpinx* and *Eupleura*. U.S. Fish Wildlife Serv., Spec. Sci. Rept. 148. 149 p.

Chadwick, G. H. 1906. Shells of Prince Edward Island. Nautilus 19: 103–104.

Clarke, A. H., Jr., D. J. Stanley, J. C. Medcof, and R. E. Drinnan. 1967. Ancient oyster and bay scallop shells from Sable Island. Nature 125: 1146–1148.

Dawson, J. W. 1871. Report on the geological structure and mineral resources of Prince Edward Island. John Lovell of Montreal, printer. Appendix p. 50–51.

Deevey, Edward S., and Richard F. Flint. 1957. Postglacial hypsithermal interval. Science 125: 182–184.

Department of Energy, Mines and Resources. 1968. Glacial map of Canada No. 1253A. Geol. Survey of Canada. Ottawa, Ont.

Drinnan, R. E., and L. A. England. 1965. Further progress in rehabilitating oyster stocks. Fish. Res. Bd. Canada, St. Andrews. Biol. Sta, Circ. Gen. Ser. 48. 4 p.

Galtsoff, Paul S. 1964. The American oyster *Crassostrea virginica* Gmelin. U.S. Fish Wildlife Serv., Bull. 64. 480 p.

Ganong, W. F. 1887. The marine Mollusca of New Brunswick. Bull. Nat. Hist. Soc. N.B. 6: 17–61.

1889. The economic Mollusca of Acadia. Bull. Nat. Hist. Soc. N.B. 8: 1–116.

1890a. Bibliographical and critical notice of Willis's published papers. Proc. Trans. N.S. Inst. Nat. Sci. 7: 412–428.

1890b. Southern invertebrates on the shores of Acadia. Trans. Roy. Soc. Canada 8: 167–185.

1890c. Zoological notes. Bull. Nat. Hist. Soc. N.B. 9: 46–59.

Ingalls, R. A., and A. W. H. Needler. MS, 1939. Preliminary report on shore mollusc resources of the Northumberland Strait coast of Nova Scotia. Fish. Res. Bd. Canada, MS Rept. 161. 16 p.

MS, 1940. A report on shore mollusc resources of the Northumberland Strait coast of Nova Scotia. Fish. Res. Bd. Canada, MS Rept. 333. 13 p.

Ives, Charles. 1907. Some notes on the land, fresh water, and marine Mollusca of Prince Edward Island. P.E.I. Agriculturist, March, 1907.

Kindle, E. M. 1916. Bottom control of marine faunas as illustrated by dredging in the Bay of Fundy. Am. J. Sci. 41: 449–461.

Lauzier, L. M., and A. Marcotte. 1967. Climat marin dans les secteurs côtiers du sud-ouest du golfe Saint-Laurent. Actualités Marines 11: 15–19.

Medcof, J. C. 1961. Oyster farming in the Maritimes. Bull. Fish. Res. Bd. Canada 131. 157 p.

Medcof, J. C., A. H. Clarke, Jr., and J. S. Erskine. 1965. Ancient Canadian east-coast oyster and quahaug shells. J. Fish. Res. Bd. Canada 22: 631–634.

Medcof, J. C., and E. I. Morrison. MS, 1943. Report on 1943 investigations. Fish. Res. Bd. Canada, MS Rept. 370. 65 p.

Merrill, A. S., K. O. Emery, and M. Rubin. 1965. Ancient oyster shells on Atlantic continental shelf. Science 147: 398–400.

Moore, G. MS, 1966. An investigation of the possibilities of seed oyster production in Great Bay, New Hampshire. MS Quart. Progr. Rept. to U.S. Bur. Comm. Fish. August 1966. 6 p.

Robertson, A. D. 1915. First report on the "barren oyster bottoms" investigation, Richmond Bay, P.E.I. Contrib. Canadian Biol. 1914–1915: 55–71.

Smith, H. I. 1929. The archaeology of Merigomish Harbour, Nova Scotia. Natl. Museum Canada Bull. 47: 5–104.

Stafford, J. 1912. On the fauna of the Atlantic coast of Canada. Second report — Malpeque, 1903–04. Contrib. Canadian Biol. 1906–1910: 37–44.

Verrill, A. E. 1873. Report upon the invertebrate animals of Vineyard Sound and the adjacent waters. Rept. U.S. Fish. Comm. (1871 and 1872): 295–778.

Whiteaves, J. F. 1901. Catalogue of the marine invertebrates of eastern Canada. Geol. Surv. Canada. Ottawa, Ont. 271 p.

Winkley, H. W. 1888. Mollusks found in the oyster beds of Cocagne, N.B. Bull. Nat. Hist. Soc. N.B. 7: 69–71.

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Phytoplankton Nutrient Enrichment Experiments off Baja California and in the Eastern Equatorial Pacific Ocean[1]

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THOMAS, WILLIAM H. 1969. Phytoplankton nutrient enrichment experiments off Baja California and in the eastern equatorial Pacific Ocean. J. Fish. Res. Bd. Canada 26: 1133–1145.

Natural phytoplankton populations were enriched with various nutrients both singly and as mixtures. After 4 or 5 days of incubation in natural light, the effects of enrichment were assessed by measuring the rate of ^{14}C uptake or changes in chlorophyll. Off Baja California, in June 1964, September 1965, and November 1966, increases in chlorophyll or ^{14}C uptake occurred in treatments containing added nitrate or trace metals. In February 1967, three enrichment experiments done in nutrient-poor water well offshore and both north and south of the Equator showed that nitrogen was the most likely limiting nutrient. In two experiments in equatorial upwelling water, no nutrient appeared to limit phytoplankton growth since chlorophyll increases were nearly as great in controls containing no enrichment as in treatments containing a complete mixture.

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INTRODUCTION

ENRICHMENT OF natural phytoplankton populations with nutrients, followed by assessment of effects on photosynthesis or growth, has proved to be a useful way to discover which nutrients limit these processes. In the Sargasso Sea, Ryther and Guillard (1959) showed that a trace metal mixture (including Fe) was most important in increasing short-term photosynthetic rates, and that silicate was also important. Menzel and Ryther (1961) added separate components of the metal mixture and showed that iron was the most important metal in experiments lasting 24 hr, but thereafter nitrogen and phosphorus were also needed. Similar effects of iron were also found in the tropical Indian Ocean by Tranter and Newell (1963). Further Sargasso Sea experiments (Menzel et al., 1963) confirmed the earlier observations; showed that the effect of iron could be duplicated by aluminium; and showed that the species composition of the final phytoplankton population depended upon the enrichment supplied.

Nutrient enrichment methods have also been applied to freshwater populations (for a review, see Goldman, 1965). Perhaps the most interesting result to date is the identification of molybdenum as a limiting factor in Castle Lake, California (Goldman, 1960).

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[1] Contribution from the Scripps Institution of Oceanography, University of California, San Diego, Calif., USA.

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During various cruises in the eastern tropical Pacific Ocean, I have performed similar experiments. One set of data from the eutrophic Costa Rica Dome (Thomas, 1959) showed that nitrogen and phosphorus were not limiting; and that silicate, trace metals, and unknown components of soil extract were likely limiting nutrients for diatom growth. Other nutrients were not tested.

Later experiments were generally inconclusive probably because incubations were for periods less than 24 hr and were done in artificial light.

This paper reports the results of several enrichment experiments carried out with natural illumination and prolonged incubation, and performed off Baja California and in the eastern equatorial Pacific Ocean. It will be shown that the most important limiting nutrient in oligotrophic water in these areas is nitrogen, although secondary effects are also attributed to trace metals in experiments made with water taken off Baja California.

METHODS

Figure 1 shows the locations of the Baja California experiments. Five experiments were carried out during three cruises: TO-64-1 (June 1964), TO-65-1 (September 1965), and TO-66-1 (November 1966).

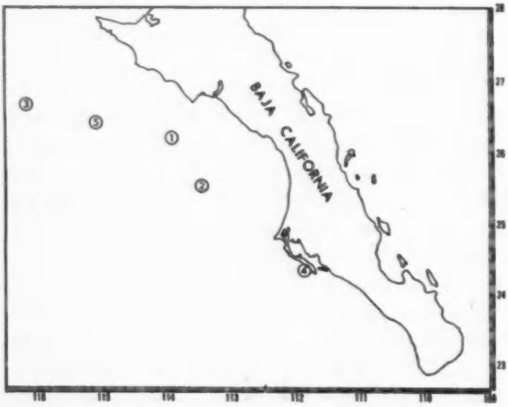

FIG. 1. Locations of Baja California enrichment experiments.

The locations of the experiments performed in the eastern equatorial Pacific Ocean are shown in Fig. 2. Five experiments were performed during the first EASTROPAC survey cruise aboard R/V *Argo* in January, February, and March 1967. Also shown in Fig. 2 are zones of high-nutrient, near-surface water.

For each experiment, near-surface sea water was collected by lowering a plastic 55-liter aspirator bottle to 10-m depth. The top of the bottle was open so that water was collected as the bottle was lowered from the surface to 10 m. It is estimated that about half of the bottle was filled during the lowering and the remainder was collected at 10 m. After filling, the bottle was raised and the water was drained out from the bottom tubulation through a plastic hose into 20-liter flat plastic bottles — these are commonly called "jerrycans." Several lowerings of the aspirator bottle were required to fill the set of jerrycans, and the water was distributed among

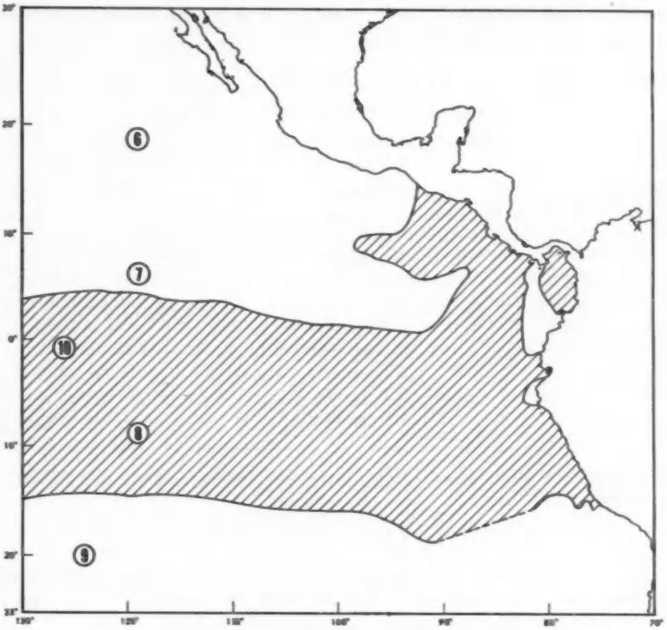

FIG. 2. Locations of enrichment experiments in the equatorial eastern Pacific Ocean. Shaded areas are zones where near-surface nutrients are high (NO_3–N $\geq$ 1.0 μg-at/liter).

the jerrycans so that each received water from each lowering. The jerrycans were filled to a volume of 17.5 liters.

Different nutrient enrichments were then added to each jerrycan. The enrichments were added as single enrichments or as mixtures of enrichments; in most experiments the mixtures consisted of complete enrichments minus single nutrients. The concentrations of these enrichments are shown in Table 1. Concentrations of nitrate, phosphate, and silicate were chosen that would be comparable to those in deep water. With the exception of manganese and cobalt, concentrations of trace metals were such that little would be added to that already present in sea water, and possible toxicities might thus be avoided. Vitamins were added at levels commonly employed in media used to culture marine phytoplankton.

After enrichment, each jerrycan was stoppered with a silicone rubber stopper, enclosed in a white cotton bag, and laid flat on the deck of the ship. The wall of the container and the cotton bag reduced the light intensity inside each jerrycan to 30% of that incident on the deck. The jerrycans were then incubated for 4 or 5 days in natural daylight. During incubation, surface sea water was sprayed on the jerrycans to keep them cool. This was not completely effective; in the tropics, temperatures inside the jerrycans reached 30 C even though the surface seawater temperature was 27 C. This temperature increase is not great and it is not believed that the increase would alter the nutritional results reported herein.

In the first experiment, the effects of enrichments were assessed by further incubation with ^{14}C (Steemann-Nielsen, 1952). Aliquots of 100 ml from each jerrycan were placed in clear glass bottles, 10 μc of $Na_2{}^{14}CO_3$ were added, and the bottles were incubated at an illuminance of 1200 ft-c (supplied by fluorescent lamps) and a temperature of 19 C for 3 hr. A set of aliquots was also

TABLE 1. Final concentrations of nutrients added to enrichment experiments.

Enrichments	Concentration added	Concentration in sea water[a]
KNO_3	25 μM	0–40 μM
K_2HPO_4	2.5 μM	0–4.0 μM
Na_2SiO_3	5 μM	0–120 μM
Fe (as NaFeEDTA)	0.2 μM	0.18 μM
EDTA	0.2 μM	–
Mn (as $MnCl_2$)	10 μg/liter	2 μg/liter
Zn (as $ZnSO_4$)	1 μg/liter	10 μg/liter
Mo (as Na_2MoO_4)	0.5 μg/liter	10 μg/liter
Co (as $CoCl_2$)	0.5 μg/liter	0.1 μg/liter
Cu (as $CuSO_4$)	0.2 μg/liter	3 μg/liter
Vitamin B_{12}	0.5 μg/liter	0.0001–0.2 μg/liter
Biotin	0.5 μg/liter	0.0006–0.003 μg/liter
Thiamin	100 μg/liter	0.003–0.5 μg/liter

[a] Taken from data of Goldberg (1965), Provasoli (1963), Carlucci and Silbernagel (1966, 1967), and Harvey (1955).

incubated with ^{14}C in darkness. After incubation with ^{14}C, the aliquots were filtered through membrane filters that were fumed with HCl and dried. The radioactivity on each filter was assayed ashore with a Nuclear-Chicago D-47 Geiger counter.

In all the other experiments, enrichment effects were assessed by measuring changes in chlorophyll in each jerrycan by in vivo fluorescence (Lorenzen, 1966). This method has been recently criticized by Strickland (1968) who showed that the factor relating chlorophyll to fluorescence can vary considerably and that strict proportionality does not hold at chlorophyll concentrations above 4 mg/m^3. In the present experiments, a mean factor was determined from many calibrations during each cruise and only in some of the treatments containing complete mixtures are chlorophyll concentrations greater than 4 mg/m^3. Nevertheless, the concentrations reported should not be accepted as absolute values; rather, the relative differences within each experiment should be considered and only values differing by a factor of two from a control treatment should be considered significant. In Tables 2–7, treatments that indicated stimulation by a given nutrient as compared with a control treatment are indicated by a + sign; inhibitory nutrients are indicated by a – sign. The in vivo method is advantageous in that it is rapid and can easily be used aboard the ship; results can be obtained quickly without difficulty, and experiments can then be set up during a cruise that take into consideration the results of previous experiments during the same cruise. Since the jerrycans were not sampled each day, it is not possible to state whether the observations represent effects on *growth rate* or upon *final yield* of algal material.

During the first two Baja California cruises the following phytoplankton nutrients were measured manually in water samples before enrichment and in samples from Nansen bottle casts taken at numerous stations: nitrate (Mullin and Riley, 1955b), ammonia (Richards and Kletsch, 1964), nitrite (Bendschneider and Robinson, 1952), phosphate (Murphy and Riley, 1962), and silicate (Mullin and Riley, 1955a). Samples were analyzed at sea as they were collected or were frozen and analyzed ashore.

During the last Baja California cruise and the EASTROPAC cruise, nutrients other than ammonia were analyzed at sea with the Technicon Autoanalyzer using methods developed by Armstrong et al. (1967). Ammonia was measured in frozen samples by the manual method.

RESULTS

BAJA CALIFORNIA EXPERIMENTS

Table 2 shows the results of the first experiment (June 1964), in which the responses of the population to enrichment were measured by ^{14}C uptake. Enrichment with nitrate or the complete mixture resulted in a threefold increase in uptake as compared with the unenriched control treatment; responses due to other single additions were zero or slight. ^{14}C uptake was greater in treatments containing nitrate plus phosphate than in the complete mixture or in the nitrate treatment. This suggests that uptake in the complete treatment may have been inhibited by enrichments other than nitrate or phosphate. However, the radioactive filter pad from the complete treatment was visibly more green than filter pads from nitrate plus phosphate treatments, and it is possible that the inhibition is an artifact in which the radioactive count is reduced by algal self-absorption of the radioactivity. This experiment definitely indicates that nitrogen was the most important limiting nutrient in these waters in June 1964. However, when nitrate is available, additional phosphate results in an increase in photosynthesis.

TABLE 2. Effects of nutrient enrichment on ^{14}C uptake in a seawater sample collected off Baja California in June 1964. Experiment 1: station 6; cruise TO-64-1; 26°16′N, 113°52′W; 5-days' incubation in natural light, followed by addition of 10 μc $Na_2{}^{14}CO_3$ to 100-ml aliquots and further incubation for 3 hr at 1200 ft-c illuminance and 19 C.

Initial nutrient concentrations (*μg-at/liter*)	Enrichment	^{14}C uptake count/min[a]
NO_3–N = 0.1[b]	None	4,378
NH_4–N = 0.40[b]	Complete nutrient mixture	12,579+
NO_2–N = 0.20	+NO_3	11,022+
PO_4–P = 0.70	+PO_4	5,198
SiO_4–Si = 4.3	+SiO_3	4,848
	+Fe	5,501
	+Trace metals	4,001
	+Vitamins	3,965
	+NO_3+PO_4	27,432+
	+NO_3 + PO_4 + Fe	27,485+

[a] Corrected for uptake in darkness.
[b] NO_3–N and NH_4–N concentrations are taken from station 8 (25°36′N, 113°47′W).

In September 1965, similar results (Table 3) were obtained using chlorophyll changes to assess the response to enrichment. Again nitrogen was the most important limiting nutrient. Phosphate, or iron plus phosphate, in addition to nitrate did not increase the chlorophyll concentration over that in the treatment enriched only with nitrate. Indeed the responses in these three treatments agreed quite well suggesting that identical treatments would also have given similar results if replication had been possible. Chlorophyll increased greatly when the population was enriched with a complete mixture

TABLE 3. Effects of nutrient enrichment on the chlorophyll concentration of a seawater sample collected off Baja California in September 1965. Experiment 2 (from Thomas, 1967): station 60; cruise TO-65-1; 25°33.3′N, 113°24.5′W; initial chlorophyll concentration, 0.11 mg/m³; 4-days' incubation in natural daylight.

Initial nutrient concentrations (*µg-at/liter*)	Enrichment	Chlorophyll/m³ (*mg*)
NO_3–N = 0.0	None	0.06
NH_4–N = 0.55	Complete nutrient mixture	9.07+
NO_2–N = 0.03	+NO_3	0.43+
PO_4–P = 0.42	+PO_4	0.06
SiO_4–Si = 1.3	+SiO_3	0.03
	+Vitamins	0.05
	+Fe	0.04
	+Trace metals	0.24+
	+NO_3 + PO_4	0.38+
	+NO_3 + PO_4 + Fe	0.41+

suggesting that if nitrate, phosphate, and iron are available, other nutrients then become limiting. There was also an increase in chlorophyll concentration after enrichment with a trace metal mixture. This experiment was previously reported (Thomas, 1967) but is included herein for comparison with other Baja California experiments.

Nitrogen limitation was again evident in November 1966 both in offshore waters (experiment 3, Table 4) and at a neritic station (experiment 4, Table 4). Such limitation was particularly shown when nitrogen was deleted from the complete mixture. However, when only trace metals were added to both inshore and offshore water, as much chlorophyll was synthesized as when nitrogen alone was added; deletion of trace metals from the complete mixture also reduced the amount of chlorophyll synthesis. Inhibition by phosphate, iron, EDTA, and vitamins was evident in experiment 4 when these nutrients were deleted from the complete mixture in that, upon deletion, chlorophyll concentrations were increased.

Late in November 1966, we made a further experiment (experiment 5) in which the components of the trace metal mixture were separated, and added singly or deleted successively from a complete mixture. Nitrogen treatments were also included and had the greatest effect. Otherwise the experiment was inconclusive; we were unable to identify which trace metal in the mixture was active in promoting chlorophyll synthesis.

EXPERIMENTS IN THE EASTERN EQUATORIAL PACIFIC OCEAN

During January–March 1967, the eastern equatorial Pacific Ocean was surveyed by several ships as the initial part of Expedition EASTROPAC. The outermost survey lines were traversed by R/V *Argo* along 119°W and 126°W and ranging from 29°N to 20°S on both longitudinal traverses. Water in the euphotic layer was nutrient-poor from 20°N to 4°N and from 15°S to 20°S. In a zone from 4°N to 15°S, nutrients increased.

TABLE 4. November 1966. Effects of nutrient enrichment on chlorophyll concentrations of seawater samples collected off Baja California, cruise TO-66-1.

	Experiment no.	
	3	4
Initial data		
Station	15 (offshore)	50 (inshore)
Lat	26°38.5′N	24°19.0′N
Long	116°09.0′W	111°48.0′W
Chlorophyll concentration (*mg/m*^{3})	0.65	0.54
Nitrate concentration (*µg-at/liter*)	0.0	0.0
Ammonia concentration (*µg-at/liter*)	0.35	0.35
Nitrite concentration (*µg-at/liter*)	0.00	0.02
Phosphate concentration (*µg-at/liter*)	0.39	0.42
Silicate concentration (*µg-at/liter*)	1.6	2.7
Period of incubation (*days*)	5	4
Enrichment	Chlorophyll/m^3 (*mg*)	
None	0.24	0.31
+NO$_3$	0.46+	0.39+
+PO$_4$	0.28	0.24
+SiO$_3$	0.20	0.24
+Fe	0.31	0.31
+EDTA	0,12	0.16
+Trace metals	0.43+	0.43+
+Vitamins	0.16	0.24
Complete	2.01	11.2
-NO$_3$	0.28+	0.46+
-PO$_4$	1.93	16.8–
SiO$_3$	1.63	13.6
−Fe	1.07+	18.4−
−EDTA	2.44	16.4−
−Trace metals	1.18+	5.6+
−Vitamins	1.71	16.4

Two enrichment experiments (experiments 6 and 7, Table 5) were performed with northerly nutrient-poor water. Both indicated that nitrogen was the most important limiting nutrient, as evidenced by strong responses to single additions of nitrogen or to deletion of this nutrient from the complete mixture. Silicate was somewhat stimulatory in experiment 6, and in experiment 7 both silicate and phosphate were important when nitrogen was present (see results of deletion of silicate and phosphate). Iron, trace metals, and vitamins were inhibitory to complete treatments in experiment 7.

Similar strong evidence for primary limitation by nitrogen was found in southerly nutrient-poor water (experiment 9, Table 6). Only nitrogen addition resulted in a great increase in chlorophyll when nutrients were added singly, and deletion of this nutrient from a complete mixture caused a large decrease

TABLE 5. January–February 1967. EASTROPAC expedition. Effects of nutrient enrichment on chlorophyll concentrations of seawater samples from the tropical Pacific Ocean — northerly nutrient-poor water.

	Experiment no.	
	6	7
Initial data		
Station	11.030	11.068
Lat	18°35.5′N	6°05.0′N
Long	119°00.0′W	118°51 0′W
Chlorophyll concentration (*mg/m*^{3})	0.12	0.13
Nitrate concentration (*μg-at/liter*	0.0	0.0
Ammonia concentration (*μg-at/liter*	0.68	0.68
Nitrite concentration (*μg-at/liter*)	0.00	0.00
Phosphate concentration (*μg-at/liter*)	0.29	0.26
Silicate concentration (*μg-at/liter*)	3.5	2.1
Period of incubation (*days*)	5	4
Enrichment	Chlorophyll/m^3 (*mg*)	
None	0.05	0.04
+NO$_3$	0.19+	0.33+
+PO$_4$	0.02	0.03
+SiO$_3$	0.10+	0.05
+Fe	0.01	0.05
+EDTA	0.05	0.04
+Trace metals	0.05	0.05
+Vitamins	0.03	0.05
Complete	0.76	1.89
−NO$_3$	0.05+	0.07+
−PO$_4$	0.98	0.31+
−SiO$_3$	0.85	0.54+
−Fe	0.73	3.77−
−EDTA	1.00	2.20
−Trace metals	0.59	3.28−
−Vitamins	0.55	5.23−

in chlorophyll. When nitrogen was present, deletion of phosphate also caused a decrease in chlorophyll concentration, but this decrease was not so great as that resulting from nitrogen deletion.

In nutrient-rich water from the equatorial upwelling area, the results of two enrichment experiments (experiments 8 and 10, Table 7) were not so clear as the results in nutrient-poor water; limitation by any one nutrient was not found. Single additions of trace metals and vitamins were stimulatory in experiment 8, and nitrogen stimulated chlorophyll synthesis in experiment 10. Deletion treatments were indicative of inhibition by iron in experiment 8 and by iron, EDTA, trace metals, and vitamins in experiment 10.

In nutrient-poor water, chlorophyll did not increase when no nutrients were added whereas in these last two experiments in nutrient-rich water,

TABLE 6. February 1967. EASTROPAC expedition. Effects of nutrient enrichment on chlorophyll concentrations of seawater samples from the tropical Pacific Ocean — southerly nutrient-poor water. Experiment 9: station 11.185; 20°01.0'S, 124°12.0'W; initial chlorophyll concentration, 0.04 mg/m^3; 5-days' incubation.

Initial nutrient concentrations (*µg-at/liter*)	Enrichment	Chlorophyll/m^3 (*mg*)
NO_3–N = 0.0	None	0.10
NH_4–N = 0.18	+NO_3	0.30+
NO_2–N = 0.00	+PO_4	0.11
PO_4–P = 0.22	+SiO_3	0.03
SiO_4–Si = 0.9	+Fe	0.11
	+EDTA	0.07
	+Trace metals	0.13
	+Vitamins	0.10
	Complete	0.84
	—NO_3	0.12+
	—PO_4	0.34+
	—SiO_3	0.67
	—Fe	0.53
	—EDTA	0.88
	—Trace metals	0.60
	—Vitamins	1.08

chlorophyll increased nearly 10-fold over the initial chloropyhll value when no nutrients were added. This suggests that the populations in rich water were not very nutrient-limited at all. Chlorophyll increases, upon single additions of nutrients, or decreases, upon their deletion, were slight, as compared to relative changes within experiments with nutrient-poor water; this lack of marked changes also suggests that the populations in nutrient-rich water were not nutrient limited.

DISCUSSION

Off Baja California, coastal upwelling occurs in the late spring and early summer. Nutrient-enriched upwelled water extends offshore and processes of nutrient uptake deplete the water significantly in a matter of days (Longhurst et al., 1967). By autumn, surface waters of the whole area, both inshore and offshore, are nutrient-poor. The distribution of nutrients off southern Baja California has been recently investigated during Tuna Oceanography cruises and will be the subject of another paper (Thomas, unpublished data). It will suffice to note here that the five Baja California enrichment experiments report results obtained from nutrient-poor water. (See nutrient data in Tables 2–4.)

Nitrogen is clearly the most important limiting nutrient in these five experiments, and this result corresponds to the low nitrate and ammonia levels found in these waters. Trace metal effects are secondary and it was not

TABLE 7. February–March 1967. EASTROPAC expedition. Effects of nutrient enrichment on chlorophyll concentrations of seawater samples from the equatorial Pacific Ocean — nutrient-rich water.

	Experiment	
	8	10
Initial data		
Station	11.140	11.254
Lat	8°41.2′S	0°44.8′S
Long	119°00.0′W	126°01.7′W
Chlorophyll concentration (*mg/m^3*)	0.14	0.30
Nitrate concentration (*μg-at/liter*)	6.6	4.5
Ammonia concentration (*μg-at/liter*)	0.54	0.41
Nitrite concentration (*μg-at/liter*)	0.00	0.00
Phosphate concentration (*μg-at/liter*)	0.69	0.53
Silicate concentration (*μg-at/liter*)	4.0	5.1
Period of incubation (*days*)	4	4
Enrichment	Chlorophyll/m^3 (*mg*)	
None	1.37	2.50
+NO_3	1.79	3.77+
+PO_4	1.06	2.12
+SiO_3	1.54	3.28
+Fe	0.94	2.41
+EDTA	2.55+	2.46
+Trace metals	1.62	1.14
+Vitamins	2.57+	2.41
Complete	1.96	1.84
−NO_3	1.12	1.08
−PO_4	1.88	1.37
−SiO_3	1.30	3.50
−Fe	5.38−	>10.0−
−EDTA	1.08	>10.0−
−Trace metals	1.00	4.09−
−Vitamins	1.61	6.18−

possible (tried in only one experiment) to identify any one trace metal as especially limiting. Enrichment with nutrients other than nitrogen or trace metals resulted in an inhibitory response or else enrichment was without effect.

In the whole eastern tropical Pacific Ocean, nitrogen deficiency would be expected over large areas merely from a consideration of nutrient analyses of near-surface water. Such deficiency was suggested in previous papers (Thomas 1966, 1967), and the nutrient data for the region have now been greatly increased because of numerous analyses done as a part of the EASTROPAC expeditions. EASTROPAC is a study of the physical, chemical, and biological oceanography of the area from 20°N to 20°S and extending from the American Coast west to 126°W. Nutrients were measured in the upper 300 m, and by making cruises every 2 months over a period of nearly 18 months, a picture of both areal and seasonal distributions of nutrients will be obtained when data analysis is completed.

Maps of the horizontal distribution of nitrate, silicate, and phosphate at 10-m depth during the first four-ship EASTROPAC survey have been drawn up (Thomas, unpublished data). These show several zones where nutrients are high (see Fig. 2): the Costa Rica Dome (probably centered at 9°N and 88°W but extending perhaps as much as 10° westward); an equatorial nutrient-rich zone ranging from 4°N southwards to 15°S and from 92° to 126°W; the Peru Current with high-nutrient water extending northerly off Ecuador; and the Panama Bight.

Within the equatorial nutrient-rich zone, 10-m nitrate–N concentrations range up to 9 μg-at/liter, phosphate–P to 0.9 μg-at/liter and silicate–Si to 6 μg-at/liter. In the nutrient-poor areas to the north and south of the equatorial upwelling zone, nitrate–N cannot generally be detected in 10-m water samples, but phosphate and silicate are always present. PO_4–P concentrations range from 0.1 to 0.4 μg-at/liter, and SiO_4–Si concentrations range from 0.5 to 3 μg-at/liter. Nitrite is nearly always absent from 10-m samples. Ammonia-N is always present in both nutrient-rich and nutrient-poor zones at concentrations <1.0 μg-at/liter. In rich equatorial water ammonia cannot be a significant nitrogen source for phytoplankton since nitrate levels are high, but in nutrient-poor water the presence of ammonia may alleviate an otherwise extreme nitrogen deficiency (Thomas, 1966).

The five EASTROPAC enrichment experiments were done with both nutrient-poor and nutrient-rich water. In both northern and southern nutrient-poor water, the primary nutrient limiting the population was nitrogen, and these results agree with what we might expect from the measured distribution of nitrate–N. When nitrogen is present and phosphate, silicate, iron, or trace metals are deleted, then these latter nutrients become limiting. However, the primary limitation is that due to lack of nitrogen. In contrast to Baja California experiments, trace metals were without effect when added singly and only of secondary importance when deleted.

In nutrient-rich equatorial water, nitrogen enrichment increased chlorophyll but was not the only enrichment that had this effect. In experiment 8, vitamins or EDTA strongly increased chlorophyll, nearly doubling the concentration; however, these effects did not occur in the other experiment (no. 10) in rich water. Certain treatments inhibited chlorophyll synthesis — particularly iron, EDTA, trace metals, or vitamins. Such inhibition was especially evident from results of treatments where these nutrients were deleted.

Possible nutrient limitations in nutrient-rich water were not so clearly evident as those in nutrient-poor water. Indeed, the population in rich water was probably not nutrient deficient at all, for chlorophyll increased nearly 10-fold over the initial value in treatments to which no nutrients were added. It seems probable that phytoplankton populations in such water are not controlled by nutrient limitation, but more likely by grazing or sinking pro-processes.

During the last 15 years, there has been an increasing emphasis placed on vitamins as possible regulators of phytoplankton in the sea. This emphasis has been the result of many investigations of pure marine phytoplankton cultures in which a majority of those algae studied have been shown to require one or another vitamin. Such requirements have been most recently listed by Provasoli (1963). However, the present experiments do not support the hypothesis that vitamins control the ecology of the oceanic phytoplankton, at least in the eastern tropical Pacific Ocean. Vitamins were stimulatory in only one experiment out of 10 when added singly (experiment 8, Table 7), and this was in nutrient-rich equatorial water. Otherwise vitamins were without effect or were inhibitory.

Enrichment experiments in the Sargasso Sea (Ryther and Guillard, 1959; Menzel and Ryther, 1961; and Menzel et al., 1963), or in the Indian Ocean (Tranter and Newell, 1963), have implicated iron as a primary controlling factor in short-term (1–3 days) experiments, but in longer experiments often nitrogen plus phosphorus enrichment alone sufficed to stimulate phytoplankton growth. The present experiments are not directly comparable to the other work, because incubation was for at least 4 days. However, in no case was enrichment with iron alone stimulatory. Deletion of this nutrient inhibited chlorophyll synthesis in two experiments, but iron never had the consistent effect (from experiment to experiment) that nitrogen had.

From all of these experiments and from nutrient determinations, a picture is emerging of the eastern tropical Pacific Ocean as an area containing desertlike zones in which nutrient levels (especially nitrate) are very low, and in which phytoplankton are particularly controlled by nitrogen. However, there are also large areas of seasonal upwelling (such as off Baja California), of nonseasonal upwelling (the Costa Rica Dome), and of upwelling plus advection from the Peru Current (the nutrient-rich equatorial area) in which phytoplankton are probably not nutrient deficient at all.

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REFERENCES

ARMSTRONG, F. A. J., C. R. STEARNS, AND J. D. H. STRICKLAND. 1967. The measurement of upwelling and subsequent biological processes by means of the Technicon Autoanalyzer and associated equipment. Deep-Sea Res. 14: 381–389.

BENDSCHNEIDER, K., AND R. J. ROBINSON. 1952. A new spectrophotometric method for the determination of nitrite in sea water. J. Marine Res. 11: 87–96.

Carlucci, A. F., and S. B. Silbernagel. 1966. Bioassay of sea water II. Methods for the determination of concentrations of dissolved vitamin B_1 in sea water. Canadian J. Microbiol. 12: 1079–1089.

1967. Bioassay of sea water IV. The determination of dissolved biotin in sea water using C^{14} uptake by cells of ***Amphidinium carteri***. Canadian J. Microbiol. 13: 979–986.

Goldberg, E. D. 1965. Minor elements in sea water, p. 163–196. *In* J. P. Riley and G. Skirrow [ed.] Chemical oceanography. Vol. 1. Academic Press, Inc., New York.

Goldman, C. R. 1960. Molybdenum as a factor limiting primary productivity in Castle Lake, California. Science 132: 1016–1017.

1965. Micronutrient limiting factors and their detection in natural phytoplankton populations, p. 121–135. *In* Primary productivity in aquatic environments. Mem. Ist. Ital. Idrobiol. 18 (suppl.) University of California Press, Berkeley, Calif.

Harvey, H. W. 1955. The chemistry and fertility of sea waters. Cambridge University Press, London, England. 224 p.

Longhurst, A. R., C. J. Lorenzen, and W. H. Thomas. 1967. The role of pelagic crabs in the grazing of phytoplankton off Baja California. Ecology 48: 190–200.

Lorenzen, C. J. 1966. A method for the continuous measurement of *in vivo* chlorophyll concentration. Deep-Sea Res. 13: 223–227.

Menzel, D. W., E. M. Hulburt, and J. H. Ryther. 1963. The effects of enriching Sargasso Sea water on the production and species composition of the phytoplankton. Deep-Sea Res. 10: 209–219.

Menzel, D. W., and J. H. Ryther. 1961. Nutrients limiting the production of phytoplankton in the Sargasso Sea, with special reference to iron. Deep-Sea Res. 7: 276–281.

Mullin, J. B., and J. P. Riley. 1955a. The colorimetric determination of silicate with special reference to sea and natural waters. Anal. Chim. Acta. 12: 162–176.

1955b. The spectrophotometric determination of nitrate in natural waters, with particular reference to sea water. Anal. Chim. Acta. 12: 464–480.

Murphy, J., and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta. 27: 31–36.

Provasoli, L. 1963. Organic regulation of phytoplankton fertility, p. 165–219. *In* M. N. Hill [ed.] The sea: ideas and observations on progress in the study of the seas. Vol. 2. Interscience, New York.

Richards, F. A., and R. A. Kletsch. 1964. The spectrophotometric determination of ammonia and labile amino compounds in fresh and sea water by oxidation to nitrite, p. 65–81. *In* Y. Miyake and T. Koyama [ed.] Sugawara festival volume, recent researches in the fields of hydrosphere, atmosphere and nuclear geochemistry. Maruzen, Tokyo.

Ryther, J. H., and R. R. L. Guillard. 1959. Enrichment experiments as a means of studying nutrients limiting to phytoplankton production. Deep-Sea Res. 6: 65–69.

Steemann-Nielsen, E. 1952. The use of radio-active carbon (C^{14}) for measuring organic production in the sea. J. Conseil, Conseil Perm. Intern. Exploration Mer. 18: 117–140.

Strickland, J. D. H. 1968. Continuous measurement of *in vivo* chlorophyll; a precautionary note. Deep-Sea Res. 15: 225–227.

Thomas, W. H. 1959. The culture of tropical oceanic phytoplankton, p. 207–209. *In* Mary Sears [ed.] International oceanographic congress preprints. Am. Assoc. Advan. Sci. Washington, D.C.

1966. Surface nitrogenous nutrients and phytoplankton in the northeastern tropical Pacific Ocean. Limnol. Oceanog. 11: 393–400.

1967. The nitrogen nutrition of phytoplankton in the eastern tropical Pacific Ocean. Proc. Intern. Conf. Trop. Oceanog., Studies Trop. Oceanog. Miami 5: 280–289.

Tranter, D. J., and B. S. Newell. 1963. Enrichment experiments in the Indian Ocean. Deep-Sea Res. 10: 1–9.

Cytological Changes in the Pituitary Gland of the Adult Sockeye Salmon (*Oncorhynchus nerka*) after Gonadectomy

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McBride, J. R., and A. P. van Overbeeke. 1969. Cytological changes in the pituitary gland of the adult sockeye salmon (*Oncorhynchus nerka*) after gonadectomy. J. Fish. Res. Bd. Canada 26: 1147–1156.

Effects of gonadectomy on the cytological structure of the pituitary gland of sockeye salmon were investigated. Castration of fully grown, but sexually immature, fish appeared to prevent differentiation of any periodic acid–Schiff (PAS) positive cells. On the other hand, castration of mature sockeye induced degranulation of this cell type. These cells later disintegrated. Although the acidophil cells of the proximal pars distalis in the immature castrates showed an apparent increase in number, those in the gonadectomized ripe fish exhibited marked hyperplasia and hypertrophy. In view of the "rejuvenation," including the considerable increase in body weight that occurs after castration of sexually ripe sockeye, it is thought that the changes in the acidophils noted in this experiment may reflect an increased production of growth hormone. Where the gonadectomy was incomplete it was noted that very small remnants of gonad were able to induce, or maintain, all the external secondary characteristics of full sexual maturity. The pituitary of these fish exhibited cytological features intermediate between those of the controls and the complete castrates.

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INTRODUCTION

Robertson (1961) reported that castration of sexually immature 2-year-old kokanee (*Oncorhynchus nerka kennerlyi*) prolonged the life span of these fish and prevented the degenerative changes which normally take place at the time of reproduction (4th year). Yet such changes were noted in their 7th year; these alterations were considered to be related to the onset of senility (Robertson and Wexler, 1962a).

Castration of adult sockeye salmon (*Oncorhynchus nerka*) approaching sexual ripeness results in a cessation, and at least a partial reversal, of the changes that these fish undergo during sexual maturation (McBride et al., 1963). Indeed, fish held to full maturity and then gonadectomized resume feeding, and by the end of the 3rd month these sockeye closely resemble silver-green sexually immature fish.

For several species of Pacific salmon (genus *Oncorhynchus*), the physiological and histological changes that occur in various endocrine organs during

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sexual maturation have been well documented (Hane and Robertson, 1959; Idler et al., 1959; Robertson et al., 1961a, b; Robertson and Wexler, 1962b; Schmidt and Idler, 1962; La Roche et al., 1963; Fagerlund, 1967; van Overbeeke and McBride, 1967; McBride, 1967). We are unaware of any studies on the effect of castration of the sexually maturing adult sockeye on these histological changes. The present communication describes the cytophysiological changes taking place in the pituitary of adult sockeye after bilateral gonadectomy.

MATERIALS AND METHODS

The sockeye used in this study were migrating fish, captured at the entrance to Great Central Lake, Vancouver Island, B.C., during the first 2 weeks of July 1965 and 1966 and transported to the laboratory. At this time they were in a sexually immature condition and silver-green ("sea green"). Methods of capture, general culture procedures, and techniques for bilateral gonadectomy have been described (McBride et al., 1963). All fish were fed. Procedures used to induce sockeye to resume feeding while held in freshwater and the composition of the diet were outlined in a previous report (McBride, 1967). Temperature of the water in the aquaria ranged from 13 C (September) to 4 C (January–February).

Experiment 1 — Sockeye captured in July 1965 were held until they were nearly ripe, judged on the basis of their secondary sexual characteristics. Of these, 15 (10 males, 5 females) were gonadectomized on September 22. They were killed in small groups 4, 6, 14, and 20 weeks after the operation. A total of 23 fish served as controls; 6 of these were killed on the day of castration.

Experiment 2 — Of the fish captured during July 1966 and held until sexually mature, 12 (7 males, 5 females) were gonadectomized on October 6, and samples of these were killed 6, 15, or 35 weeks later. There were 12 controls of which 6 were killed on the day of gonadectomy. Since sockeye normally spawn between early October and late November and die within 2–3 weeks of spawning, it was necessary to use spent fish as controls for the castrates, killed after 15 weeks. No controls were available for the castrates killed after mid-December. As the results of experiments 1 and 2 were essentially the same, their data have been pooled and presented in Table 1.

Experiment 3 — In July 1966, 9 immature sockeye (6 males, 3 females) were gonadectomized 2 weeks after capture and killed 15 or 20 weeks later along with 10 controls. A total of 29 fish (16 males, 13 females) served as controls at the beginning of the experiment (Table 2).[2]

Pituitary glands were fixed in Bouin-Hollande-sublimate and embedded in Paraplast (van Overbeeke and McBride, 1967). Series of sagittal sections, cut at a thickness of 3 and 5 μ were stained by the following methods; Masson's trichrome, including ponceau de xylidine (Culling, 1957); Bargmann's chrome–alum hematoxylin–phloxin (Pearse, 1961); periodic acid–Schiff (PAS)–Orange G (Culling, 1957); Alcian blue–PAS–Orange G (McManus and Mowry, 1960).

Since evidence concerning the cellular origin of specific hormones produced in the sockeye pituitary is insufficient, a functional nomenclature is not warranted. Instead, a morphological terminology is used, based on tinctorial and histochemical properties as well as topographical characteristics of the various cell types. In the pars distalis the following cell types are designated. *PAS-positive cells*: all cells with glycoprotein-containing granules. These cells are commonly described as basophils or cyanophils. None of the techniques applied permitted a further differentiation of this category. *Acidophils*: cells in the proximal pars distalis, whose granules possess a very general affinity to acid dyes. *Fuchsinophils*: cells in the rostral pars distalis arranged in follicles. Their granules stain with some acid dyes, particularly with acid fuchsin. *Ponceau-cells*:

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[2] For reasons of convenience, in the remainder of this report the results of this last experiment will precede those of the two others.

TABLE 1. Effect of bilateral gonadectomy on external characteristics of sexually mature adult sockeye salmon, *Oncorhynchus nerka*.

Time after gonadectomy (*weeks*)	Group	No. and sex of fish	Condition of gonad at time of autopsy	External characteristics at time of autopsy
0 days	Controls	6♂, 6♀	Ripe[a]	As mature fish[b]
4	Castrates	2♂, 2♀	Absent	Fading spawning colour
	Controls	4♂, 5♀	Ripe	As mature fish
6	Castrates	6♂, 2♀	Absent in 7 fish	Silver-green
			One ♀ : 100–120 eggs in abdomen	Silver-green
	Controls	3♂, 3♀	Ripe	As mature fish
13–15	Castrates	4♂, 2♀	Absent in 4 fish	Silver-green
			One ♂: 5-g ripe testis	As mature fish
			One ♂: 1-g ripe testis	Silver-green
	Controls	4♂	Ripe	As mature fish
		1♂, 3♀	Spent[c]	As mature fish
20	Castrates	3♂, 1♀	Absent in 2 fish	Silver-green
			One ♂: 1-g ripe testis	Faint red
			One ♀ : 100–120 eggs in abdomen	Silver-green
	Controls	Not available		
35	Castrates	2♂, 3♀	Absent	Silver-green
	Controls	Not available		

[a] Gonad weight males: >3%; females: >17% (of body weight).
[b] Red spawning colour in both sexes; hooked jaws and premaxillary teeth in males.
[c] Gonads appear collapsed; in males small amount of running milt; in females small number of eggs in abdomen.

TABLE 2. Effect of bilateral gonadectomy on external characteristics of sexually immature sockeye salmon, *Oncorhynchus nerka*.

Time after gonadectomy (*weeks*)	Group	No. and sex of fish	Condition of gonad at time of autopsy	External characteristics at time of autopsy
0 days	Controls	16♂, 13♀	Unripe[a]	Silver-green
15	Castrates	6♂	Absent in 3 fish	Silver-green
			One ♂: 7.4-g ripe testis	As mature fish[b]
			One ♂: 9.2-g ripe testis	” ” ”
			One ♂: 2.7-g ripe testis	” ” ”
	Controls	3♂, 2♀	Ripe[c]	” ” ”
20	Castrates	3♀	Absent	Silver-green
	Controls	1♂, 4♀	Ripe	As mature fish

[a] Gonad weight males: <1%; females: <4% (of body weight).
[b] Red spawning colour in both sexes; hooked jaws and premaxillary teeth in males.
[c] Gonad weight males: >3%; females: >17% (of body weight).

a layer of columnar cells at the boundary of the rostral pars distalis with the neurohypophysis. They are refractory to most dyes, but stain clearly with ponceau de xylidine. *Chromophobes*: conspicuous, often large cells, confined to the proximal pars distalis and lacking in discernible granulation. *Undifferentiated cells*: groups of densely packed, small, fusiform cells forming the cores of the cell columns in the proximal pars distalis. Their cytoplasm is often slightly basophilic but lacks discernible secretory granules. "*Embryonic cells*": very small cells with spherical nuclei and very little chromophobe cytoplasm. These cells occur in small groups and are characteristically located in the uppermost parts of the cell columns adjoining the pars nervosa.

RESULTS

EFFECT OF CASTRATION ON EXTERNAL FEATURES AND BEHAVIOUR

Two months after capture, the unoperated control fish showed the characteristics of sexual maturity: red spawning colour in both sexes, and in the males elongated hooked jaws and the presence of premaxillary teeth. They displayed distinct aggressive behaviour and were found dispersed throughout the tanks. Of the nine sockeye gonadectomized in July 1966 while still sexually immature, six failed to develop such features. The remaining three were not distinguishable from the controls. Autopsy revealed that these three contained small parts of ripe testis (Table 2). The remnants weighed from 0.17 to 0.35% of the total body weight (testis weight in mature males: >3%) and were found either in the extreme cranial or caudal end of the abdomen.

Castration of ripe sockeye resulted in a fading of the red colour in both males and females within 2 weeks. By the 8th week, most of the operated fish exhibited greenish backs and silvery sides and bellies, thus resembling sexually immature fish. The hooked jaws of the males did not disappear. In contrast to the controls, the castrates gradually became docile and soon were swimming in tight schools. Two of the operated males, however, retained their spawning colour and continued to exhibit signs of aggressive behaviour. On autopsy, these two males were found to contain small fragments of testis (0.05 and 0.18% of their respective body weights, Table 1). In two of the castrated females, a number of eggs present in the abdomen indicated that gonadectomy had been incomplete. But this appeared to have had no effect on the changes in external features.

PITUITARY GLAND

CONTROL FISH[3]

The pituitary glands of all fish killed in July showed the characteristics of sexual immaturity. The proximal pars distalis contained large numbers of undifferentiated cells (Fig. 1), most of them densely packed and forming the cores of the cell columns. Acidophil cells were small and abundant only in the lateral parts. The chromophobes were numerous, often very large, and in many specimens showed signs of vacuolar disintegration of the cytoplasm. PAS-positive cells could be identified in only 21 of 29 fish, and in all 21 specimens these cells were small and only sparsely granulated. The rostral pars distalis in July fish consisted chiefly of follicularly arranged columnar cells containing small fuchsinophil granules. PAS-positive cells in this area were found in nine fish only. All of these were specimens that had relatively large numbers of PAS-positive cells in the proximal pars distalis. A distinct layer of cells separated the major portion of the rostral pars distalis from the neurohypophysis.

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[3] Only a summary is given. A detailed description of the sockeye pituitary and its changes during sexual maturation has been published elsewhere (van Overbeeke and McBride, 1967). Since the effects of castration were seen only in the pars distalis, the pars intermedia and the neurohypophysis are not considered in this report.

The pars distalis of the sexually mature sockeye (late September–October) differed considerably from that in the previous group. Its proximal part consisted chiefly of acidophils and PAS-positive cells. Both were numerous, but whereas the first were not visibly larger than in immature sockeye (Fig. 2), the latter were often very large and full of granules (Fig. 3). A few of the PAS-positive cells were shrunken and had pyknotic nuclei. Chromophobes, many in an advanced state of disintegration, were seen in 3 of the 12 fish. Undifferentiated cells were generally sparse and sometimes entirely absent. The fuchsinophil cells of the rostral pars distalis revealed little change except that they were slender and their dense granulation was spread throughout the whole of the cytoplasm. In all fish, PAS-positive cells were numerous in this area (Fig. 4). The layer of columnar cells, partly delineating the proximal pars distalis, stained deeply.

The pituitaries of other mature control sockeye, killed in later stages of the experiments (November–December), were similar to those just described (Fig. 5). However, the incidence of pyknosis among PAS-positive cells in both parts of the pars distalis showed an incresae, although some of the cells of this category apparently underwent slight degranulation. Also, there was a further reduction in chromophobes and undifferentiated cells. In addition, the pituitaries of several fish killed in December, including three of the four spent sockeye, exhibited signs of deterioration: disrupted cell columns, fibrosis, and occasional hemorrhages.

GONADECTOMIZED IMMATURE FISH

In contrast to the controls, in each of the six completely castrated sockeye, PAS-positive cells were entirely absent (Fig. 6). The pars distalis in these fish was, on the whole, not unlike that in immature controls killed at the beginning of the experiment. The acidophils, however, appear to be somewhat more abundant and more deeply stained. Furthermore, in all these castrates, many of the cells that, on the basis of their morphological features and type of arrangement, would have to be considered to be undifferentiated cells, contained scattered acidophil granules. In the three males that developed external characteristics of sexual maturity and in which small remants of ripe testis were found, the pars distalis was intermediate between that of a completely gonadectomized and a fully mature fish. PAS-positive cells in both parts were numerous but definitely less abundant than in any of the mature controls. Chromophobes, often intact, were present and the proportion of undifferentiated cells was high.

GONADECTOMIZED MATURE FISH

By the end of the 1st month after castration, striking changes were evident in the proximal pars distalis. Although PAS-positive cells dominated this region in both castrates and controls, only in the castrates did this cell-type exhibit varying degrees of degranulation. Also, although pyknotic degeneration of these cells was present in all of the controls, it was rarely noted in the castrates. A second effect concerned the acidophils, which in the castrates were larger and densely granulated. Small numbers of chromophobes were seen in all the castrates but in only one of the control fish. In the rostral pars distalis of the castrates slight degranulation of PAS-positive cells was also observed.[4]

Fish killed 6 weeks after gonadectomy showed a similar, but more pronounced effect (Fig. 7). Most of the PAS-positive cells were in a state of advanced degranulation and the incidence of cells devoid of stainable granules suggests that in many cells this process had led to depletion. Such depleted cells were, however, hard to distinguish from typical chromophobes. Moreover, they apparently were subject to a mode of disintegration similar to that exhibited by chromophobe cells in maturing sockeye. Since, in the controls of this sample, chromophobes were practically absent, it seems very likely that the occurrence of these depleted cells in the castrates is indeed the consequence of a progressive degranulation of the PAS-positive cells. The acidophils were affected in the same way as those in the previous sample of castrates; they were larger than in the controls and unusually deeply stained (Fig. 8). Small quantities of undifferentiated cells were present in both castrates and controls.

In sockeye, killed 13–15 weeks after gonadectomy, PAS-positive cells were almost entirely absent (Fig. 9). Only a few small cells were located in the rostral pars distalis. Of these, a few were shrunken and contained pyknotic nuclei. In sexually mature fish, a small portion of the PAS-positive cells also underwent shrinkage and pyknosis of the nuclei. Such cells are possibly no longer susceptible to the effect of castration. In the proximal pars distalis, the chief constituents of the cell columns were acidophils and depleted cells, the first commonly peripheral, the latter concentrated in the center (Fig. 10). Although part of the depleted cells exhibited various stages of disintegration, many appeared healthy and mitotic figures were noted occasionally. None of the gonadectomized fish showed signs of unspecific histolysis, which had affected five of the eight controls. No further changes were observed in the sockeye killed 20 weeks after gonadectomy except that, of the few PAS-positive cells noted, all appeared shrunken and, although their cytoplasm was deeply stained, distinct granules were found only sporadically.

Five castrates were killed during June, 35 weeks after gonadectomy. In these fish, the category of PAS-positive cells was represented by a few

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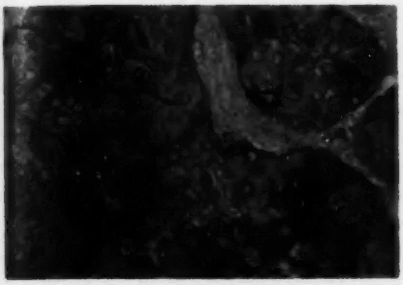
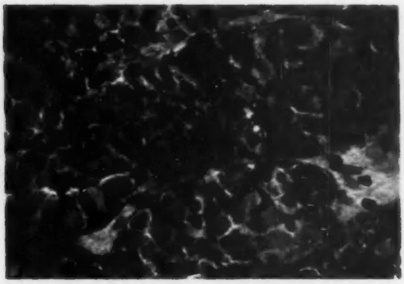
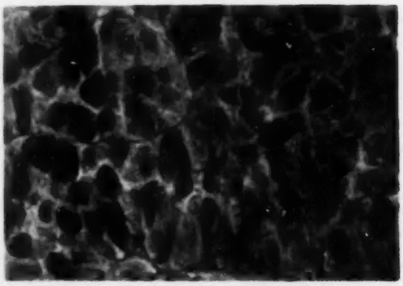
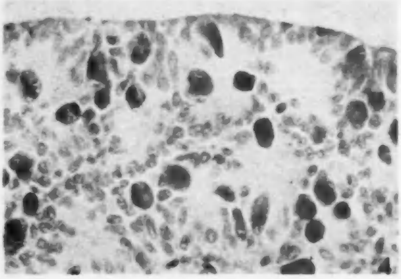
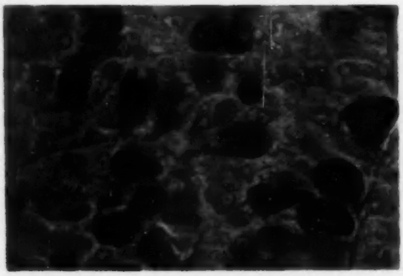
[4] An effect of gonadectomy on the epsilon cells (stained with Ponceau) was evident. Details will be reported elsewhere.











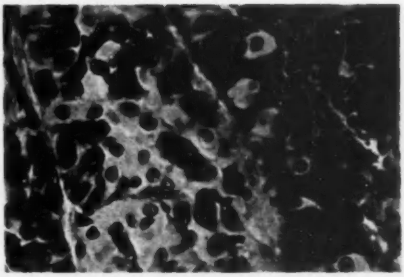


FIG. 1. Sexually immature fish (July). Proximal pars distalis near pervading neurohypophysial tissue. Small yellow cells: acidophils; larger mauve cells near wedge of neurohypophysial tissue: chromophobes; PAS-positive cells are absent. Note mass of small undifferentiated cells at lower left. Alcian blue–PAS–Orange G ×375.

FIG. 2. Sexually mature fish (October). Proximal pars distalis. Acidophil cells are red. Granules of PAS-positive cells in this technique show an affinity for light green. Just off center is a small group of undifferentiated cells. Masson trichrome. ×375.

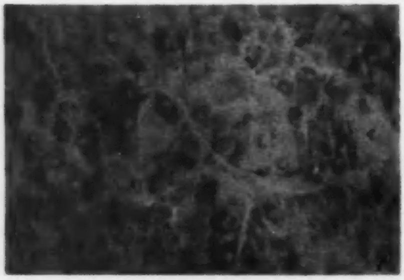
FIG. 3. Sexually mature fish (late September). Proximal pars distalis. This area consists mainly of PAS-positive cells (purple) and acidophils (yellow). Note that in the first the granules are PAS-positive, whereas the cytoplasm shows an affinity for Alcian blue. Compare with Fig. 1. Alcian blue–PAS–Orange G. ×375.

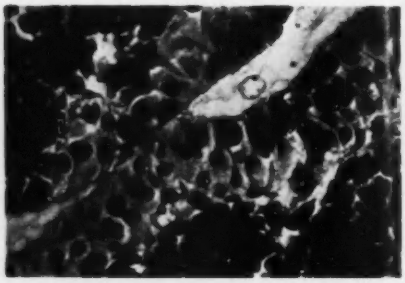
FIG. 4. Sexually mature fish (late September). Portion of rostral pars distalis, showing a number of follicularly arranged cells. Each follicle contains a few large PAS-positive cells. PAS–hemalum. ×375.

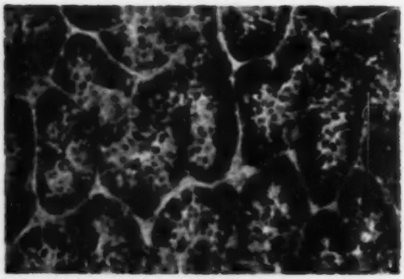
FIG. 5. Spent fish, killed just after completion of spawning (December). Cellular composition of proximal pars distalis is essentially the same as in sexually mature fish. Compare with Fig. 3. Alcian blue–PAS–Orange G. ×600.

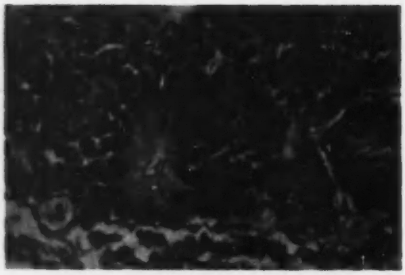
FIG. 6. Fish gonadectomized when sexually immature (July) and killed after 5 months. The proximal pars distalis consists of typical acidophils (large red cells), chromophobes, and small undifferentiated cells (upper right corner). Note that the latter are acidophilic. Light-green stained cells are entirely absent. Compare with Fig. 2. Masson trichrome. ×375.

All photographs were taken from midsagittal sections of sockeye pituitary glands.









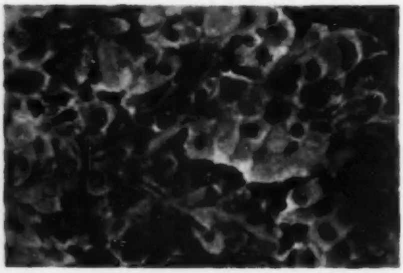


FIG. 7. Fish gonadectomized when sexually mature and killed after 6 weeks. Same part of proximal pars distalis as shown in Fig. 3 and 5. PAS-positive cells in advanced state of degranulation. Alcian blue–PAS–Orange G. ×375.

FIG. 8. Fish gonadectomized when sexually mature and killed after 6 weeks. Proximal pars distalis. Acidophils deeply stained and often large. Cells, normally staining with light green are now depleted and show loss of cytoplasm. Compare with Fig. 2. Masson trichrome. ×375.

FIG. 9. Fish gonadectomized when sexually mature and killed 15 weeks later. Same area as in Fig. 3, 5, and 7. PAS-positive cells are entirely depleted and show disintegration of cytoplasm. Acidophils are pale yellow. Alcian blue–PAS–Orange G. ×375.

FIG. 10. Fish gonadectomized when sexually mature and killed 15 weeks later. Cell columns in the proximal pars distalis show characteristic arrangement of peripheral acidophils (red) and centrally located depleted "basophils." Masson trichrome. ×150.

FIG. 11. Fish gonadectomized when sexually mature and killed 35 weeks later. Portion of rostral pars distalis. Only a few small PAS-positive cells left. Compare with Fig. 4. PAS–hemalum. ×375.

FIG. 12. Incompletely gonadectomized mature fish (approx 1 g testis tissue found at autopsy, 20 weeks after castration). "Basophils" are only partly degranulated; depleted basophils are intact and show mitotic activity. Compare with Fig. 2 and 6. Masson trichrome. × 375.

All photographs were taken from midsagittal sections of sockeye pituitary glands very small and isolated cells in the rostroventral area of the pars distalis (Fig. 11). It is of interest that this is the same area where such cells were seen to commence differentiation in normal immature sockeye in July of the previous year. The condition of the PAS-positive cells in the castrates, however, suggests that they are deteriorating remnants rather than cells in an early stage of differentiation. The general picture of the pars distalis differed from that in the previous groups of castrates in that neither typical chromophobes nor depleted PAS-positive cells were present. The proximal part consisted almost exclusively of acidophil cells, but unlike some of the gonadectomized fish killed earlier, the acidophilia was far less conspicuous.

INCOMPLETELY GONADECTOMIZED MATURE SOCKEYE

The pituitaries of those sockeye that appeared to have been incompletely gonadectomized (Table 1) showed only a limited effect of the operation. This was particularly evident in the specimens killed after 13–15 weeks (2 males) and after 20 weeks (1 male, 1 female). In each of these four fish, PAS-positive cells were abundant and, although many appeared to be in a state of advanced degranulation, the proportion of depleted cells was low (Fig. 12). The acidophils were not different from those observed in the normal mature fish. In all four, undifferentiated cells were fairly numerous but exhibited a weak acidophilia.

Typical castration cells ("signet ring cells") described in mammals and also in some teleosts (Atz, 1953; Robertson and Wexler, 1962a; Schreibman, 1964) were not found in any of the castrated sockeye. Ultimate degeneration of the degranulated cells appears to be the rule, but accumulation of depleted cells during the first months after castration indicates that disintegration lags behind degranulation. Moreover, in this stage many of these cells appeared healthy and mitoses were not uncommon.

DISCUSSION

There is a general agreement that gonad maturation in teleost fishes is under pituitary control. The literature has been reviewed by Pickford and Atz (1957), Ball (1960), and Hoar (1965, 1966). Recent studies indicate that gonadotrophic hormones primarily regulate the later stages of gametogenesis (Olivereau and Ridgway, 1962; Barr, 1963a, b; Barr and Hobson, 1964; Yamazaki, 1965; Ahsan, 1966). Although, to our knowledge, no detailed investigations on the pituitary–gonad relationship in *Oncorhynchus* species have been published, there exists ample evidence for a pituitary-controlled gonad function in this genus (Otsuka, 1956a, b; Robertson and Rinfret, 1957; Schmidt et al., 1965; Yamazaki, 1965; Ahsan, 1966). Histological and cytochemical analysis of the teleost pituitary gland indicates that gonadotrophic hormones are produced by PAS-positive cells, often identified as "basophils" or "cyanophils." It has been shown that this cell type is predominant in the pars distalis of sexually mature Pacific salmon (Robertson and Wexler, 1957, 1962b; Olivereau and Ridgway, 1962; van Overbeeke and McBride, 1967).

In the teleost pituitary gland, PAS-positive cells comprise both gonadotrophs and thyrotrophs. There is general agreement that differentiations between these cell types can be achieved only by experimental manipulation with their respective targets, i.e., gonads and thyroid gland. Our results would indicate that in the sockeye used in this study, all PAS-positive cells are gonadotrophs. Thyrotrophs, containing finely granulated cytoplasm, as were described in a number of teleosts (Olivereau, 1963a, b), could not be discerned in our material. It is of interest that Robertson and Wexler (1962b) did not identify this cell type in their study of mature Pacific salmon. Furthermore, in salmonids such cells (delta cells) are scanty and very difficult to identify (Olivereau, 1963a, b; Olivereau et al., 1964). This last finding seems to be in accordance with the reduced thyroid activity in both Atlantic and Pacific salmon at the time of reproduction (Olivereau, 1954; Robertson and Wexler, 1960; Robertson et al., 1961a). Nevertheless, the possibility cannot be excluded that in our fish some thyrotrophs were present and that these cells had undergone degranulation after gonadectomy. A similar effect of gonadectomy on the thyrotrophs has been observed by Schreibman (1964).

Whether, in teleosts, the gonads exert some control over the gonadotrophic activity in the pituitary gland is a matter of interest. A histological effect of castration on the pars distalis has been reported for a few species (Sokol, 1955; Robertson and Wexler, 1962a; Schreibman, 1964). Since, in sockeye salmon, differentiation of the pars distalis during sexual maturation is a gradual process and involves various cell types (van Overbeeke and McBride, 1967), it was to be expected that effects of gonadectomy would be variable and probably dependent on the actual condition of the pituitary gland at the time of operation. Our results indeed show such differences, but irrespective of the time of castration, the condition of the gonadotrophs appeared to be specifically affected: their development appeared to have been supressed in immature fish although they underwent rapid degranulation in mature or nearly ripe fish. Thus, it seems justified to conclude that our data provide further evidence for the existence of a feedback relationship between the pituitary and the gonads.

The proportion of acidophil cells in the proximal pars distalis of Pacific salmon shows an increase during spawning migration (Robertson and Wexler, 1962b; Olivereau and Ridgway, 1962; van Overbeeke and McBride, 1967). In both immature and mature sockeye, gonadectomy results in a considerable recruitment of acidophils, which ultimately become the predominant cell type. Although mitosis seems to contribute to an increase in their number, the results indicate that conversion of undifferentiated cells plays the major role. Acidophils have been related to the production of growth hormone. In several species of teleosts they appear to be abundant in periods of rapid growth (Olivereau and Ridgway, 1962; Olivereau, 1963b; Schreibman, 1964). Recently, Ball (1965) provided experimental evidence for a somatotropic function of this cell type in *Poecilia latipinna*. Growth induced by castration has been reported for kokanee salmon (Robertson, 1961) as well as for sockeye (McBride et al., 1963). Although no complete weight records for the castrates of the present investigation are available, there is no doubt that they, too, grew considerably. It would be in accordance with the above observations to suggest that the hypertrophy and hyperplasia of the acidophils in the castrated sockeye reflects an increased production of growth hormone.

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REFERENCES

Ahsan, S. N. 1966. Effects of gonadotropic hormones on male hypophysectomized lake chub, *Couesius plumbeus*. Canadian J. Zool. 44: 703–717.

Atz, E. H. 1953. Experimental differentiation of basophil cell types in the transitional lobe of the pituitary of a teleost fish, *Astyanax mexicanus*. Bull. Bingham Oceanog. Coll. 14: 94–116.

Ball, J. N. 1960. Reproduction in female bony fishes. Symp. Zool. Soc. London 1: 105–135.

1965. Partial hypophysectomy in the teleost poecilia: Separate identities of teleostean growth hormone and teleostean prolactin-like hormone. Gen. Comp. Endocrinol. 5: 654–661.

Barr, W. A. 1963a. The endocrine control of the sexual cycle in the plaice, *Pleuronectus platessa* L. II. The endocrine control of oogenesis. Gen. Comp. Endocrinol. 3: 205–215.

1963b. The endocrine control of the sexual cycle in the plaice, *Pleuronectus platessa* L. III. The endocrine control of spermatogenesis. Gen. Comp. Endocrinol. 3: 216–225.

Barr, W. A., and B. M. Hobson. 1964. The endocrine control of the sexual cycle in the plaice, *Pleuronectus platessa* L. IV. Gonadotropic activity of the pituitary gland. Gen. Comp. Endocrinol. 4: 608–613.

Culling, C. F. A. 1957. Handbook of histopathological technique. Butterworth & Co. (Publishers) Limited, London. 446 p.

Fagerlund, U. H. M. 1967. Plasma cortisol-concentration in relation to stress in adult sockeye salmon during the fresh water stage of their life cycle. Gen. Comp. Endocrinol. 8: 197–207.

Hane, S., and O. H. Robertson. 1959. Changes in plasma 17-hydroxycorticosteroids accompanying sexual maturation and spawning of the Pacific salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Salmo gairdnerii*). Proc. Natl. Acad. Sci. 45: 886–893.

Hoar, W. S. 1965. Comparative physiology: hormones and reproduction in fishes. Ann. Rev. Physiol. 27: 51–70.

1966. Hormonal activities of the *pars distalis* in cyclostomes, fish and amphibia, p. 242–294. *In* G. W. Harris and B. T. Donovan [ed.] The pituitary gland. Vol. 1. Butterworth & Co. (Publishers) Limited, London.

Idler, D. R., A. P. Ronalds, and P. J. Schmidt. 1959. Biochemical studies on sockeye salmon during spawning migration. VII. Steroid hormones in plasma. Canadian J. Biochem. Physiol. 37: 1227–1238.

La Roche, G., C. L. Johnson, A. N. Woodall, and L. L. Rosenberg. 1963. Iodine metabolism in adult king salmon (*Oncorhynchus tshawytscha*, Walbaum). Proc. XVI Intern. Congr. Zool. (Wash., D.C.) 2: 154.

McBride, J. R. 1967. Effects of feeding on the thyroid, kidney, and pancreas in sexually ripening adult sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada 24: 67–76.

McBride, J. R., U. H. M. Fagerlund, M. Smith, and N. Tomlinson. 1963. Resumption of feeding by and survival of adult sockeye salmon (*Oncorhynchus nerka*) following advanced gonad development. J. Fish. Res. Bd. Canada 20: 95–100.

McManus, J. F. A., and R. W. Mowry. 1960. Staining methods. Histological and histochemical. Paul B. Hoeber, Inc., New York. 423 p.

Olivereau, M. 1954. Hypophyse et glande thyroïde chez les poissons. Ann. Inst. Océanog. 29: 95–296.

1963a. Effects de la radiothyroïdectomie sur l'hypophyse de l'Anguille. Discussion sur la *pars distalis* des Téléostéens. Gen. Comp. Endocrinol. 3: 312–332.

1963b. Cytophysiologie du lobe distal de l'hypophyse des Agnathes et des poissons à l'exclusion de celle concernant la fonction gonadotrope, p. 315–329. *In* J. Benoit and C. De Lage [ed.] Cytologie de l'adénohypophyse Éditions du Centre National de la Recherche Scientifique, Paris.

Olivereau, M., G. La Roche, and A. N. Woodall. 1964. Modifications, cytologiques de l'hypophyse de la truite à la suite d'une careuce en iode et d'une radiothyroïdectomie. Ann. Endocrinol. Paris 25: 481–490.

Olivereau, M., and G. J. Ridgway. 1962. Cytologie hypophysaire et antigène sérique en relation avec la maturation sexuelle chez *Oncorhynchus* species. Compt. Rend. 254: 753–755.

Otsuka, S. 1956a. Comparative studies on the pituitary hormones of vertebrates. 4. Effects of crude LH and FSH on the ovulation of hypophysectomized newts. Endocrinol. Japon. 181–185.

1956b. On the extraction and bioassay of follicle stimulating and luteinizing substances of the salmon. Endocrinol. Japon. 3: 272–277.

Pearse, A. G. E. 1961. Histochemistry. 2nd ed. J. and A. Churchill Ltd., London. 998 p.

Pickford, G. E., and J. W. Atz. 1957. The physiology of the pituitary gland of fishes. New York Zool. Soc., New York. 613 p.

Robertson, O. H. 1961. Prolongation of the life span of kokanee salmon (*Oncorhynchus nerka kennerlyi*) by castration before beginning of gonad development. Proc. Natl. Acad. Sci. 47: 609–621.

Robertson, O. H., M. A. Krupp, C. B. Favour, S. Hane, and S. F. Thomas. 1961a. Physiological changes occurring in the blood of the Pacific salmon (*Oncorhynchus tshawytscha*). Endocrinology 68: 733–746.

Robertson, O. H., M. A. Krupp, S. F. Thomas, C. B. Favour, S. Hane, and B. C. Wexler. 1961b. Hyperadrenocorticism in spawning migratory and non-migratory rainbow trout (*Salmo gairdnerii*): Comparison with Pacific salmon (Genus *Oncorhynchus*). Gen. Comp. Endocrinol. 1: 473–484.

Robertson, O. H., and A. P. Rinfret. 1957. Maturation of the infantile testes in rainbow trout (*Salmo gairdnerii*) produced by salmon pituitary gonadotrophins administered in cholesterol pellets. Endocrinology 60: 559–562.

Robertson, O. H., and B. C. Wexler. 1957. Pituitary degeneration and adrenal tissue hyperplasia in spawning Pacific salmon. Science 125: 1295–1296.

1960. Histological changes in the organs and tissues of migrating and spawning Pacific salmon (Genus *Oncorhynchus*). Endocrinology 66: 222–239.

1962a. Histological changes in the organs and tissues of senile castrated kokanee salmon (*Oncorhynchus nerka kennerlyi*). Gen. Comp. Endocrinol. 2: 458–472.

1962b. Histological changes in the pituitary gland of the Pacific salmon (Genus *Oncorhynchus*) accompanying sexual maturation and spawning. J. Morphol. 110: 171–185.

Schmidt, P. J., and D. R. Idler. 1962. Steroid hormones in the plasma of salmon at various stages of maturation. Gen. Comp. Endocrinol. 2: 204–214.

Schmidt, P. J., B. S. Mitchell, M. Smith, and H. Tsuyuki. 1965. Pituitary hormones of the Pacific salmon. I. Response of gonads in immature trout (*Salmo gairdnerii*) to extracts of pituitary glands from adult Pacific salmon (*Oncorhynchus*). Gen. Comp. Endocrinol. 5: 197–206.

Schreibman, M. P. 1964. Studies on the pituitary gland of *Xiphophorus maculatus* (the platyfish). Zoologica 49: 217–243.

Sokol, H. W. 1955. Experimental demonstration of thyrotropin and gonadotropin activity on the adenohypophysis of the guppy, *Lebistes reticulatus* (Peters). Anat. Rec. 122: 451.

van Overbeeke, A. P., and J. R. McBride. 1967. The pituitary gland of the sockeye (*Oncorhynchus nerka*) during sexual maturation and spawning. J. Fish. Res. Bd. Canada 24: 1791–1810.

Yamazaki, F. 1965. Endocrinological studies on the reproduction of the female goldfish, *Carassius auratus* L., with special reference to the function of the pituitary gland. Mem. Fac. Fish. Hokkaido Univ. 13: 1–64.

Aquatic Insect Communities of a Small Stream on Mont St. Hilaire, Quebec

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MACKAY, ROSEMARY J. 1969. Aquatic insect communities of a small stream on Mont St. Hilaire, Quebec. J. Fish. Res. Bd. Canada 26: 1157–1183.

Weekly sampling of a small woodland stream in Quebec between March 1966 and March 1967 revealed at least 120 species of insects in sand, gravel, stones, leaves, and leaf detritus habitats. Fallen leaves were the primary food source. Characteristic communities of insects were found in each habitat. Widely distributed species were usually more abundant in one of the five habitats than in the others. Some species changed their habitats with age. Congeneric species either occupied different parts of the stream or developed at different times of the year. Linear distributions of insects were determined by the substrate type, by the extent of summer drought and the ability to avoid desiccation, and in some species by upstream flying of adult females. The species composition of each community may be characteristic of small, slow-flowing streams in deciduous forests in eastern Canada.

MACKAY, ROSEMARY J. 1969. Aquatic insect communities of a small stream on Mont St. Hilaire, Quebec. J. Fish. Res. Bd. Canada 26: 1157–1183.

Un ruisseau frais et ombragé du Québec fut étudié de mars 1966 à mars 1967. Des échantillonnages hebdomadaires ont démontré qu'il y a au moins 120 espèces d'insectes vivant dans le sable, le gravier, et les détritus de feuilles, et entre les pierres et les feuilles tombées. La chute de feuilles constituait le principal apport de nourriture. Dans chaque habitat on trouvaient des communautés·charactéristiques d'insectes bien que quelques espèces se trouvaient dans plus d'un habitat. Certaines espèces changaient d'habitat selon l'âge. Les espèces du même genre n'occupaient pas la même section du ruisseau ou bien se développaient en saisons différentes. La distribution des insectes le long du ruisseau était determinée par le type de substrat; par le degré d'assèchement durant l'été et l'aptitude à éviter le dessiccation; et dans une certaine mesure par le vol des femelles adultes. Il est possible que dans chacun de ces habitats ces espèces soient la faune typique des ruisseaux lents de forêts caduques de l'est du Canada.

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INTRODUCTION

INCREASING CONCERN over the pollution of streams and rivers has stimulated the search for plant and animal indicators of water quality (Gaufin and Tarzwell, 1956; Hynes, 1960, 1962; Patrick, 1962). But in order to measure the rate and extent of changes in stream conditions, more basic information on species in "natural" waters (Hynes, 1960) must be available. The presence or absence of a single index species is probably not as useful in revealing the condition of the water as the species composition of the stream community as a whole. Although microorganisms may eventually prove to be the most

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specific indicators, present field methods rely on a knowledge of the macrofauna, especially aquatic insects. However, although the number of described insect species is increasing, purely taxonomic studies usually do not include a description of the community from which an animal came, and the records seldom include details of the ecology and seasonal occurrence of the immature stages. The most valuable ecological investigation of stream insects in northeastern North America, that of Sprules (1947) is confined to the summer emergence of adults. No account of stream communities in this area has included all members of winter and summer faunas.

The present study of insect communities in a small woodland stream was undertaken to examine the species compositions in different habitats within the stream, including the distributions of immature stages throughout the year. The ability of many species to tolerate large amounts of allochthonous organic material in the form of autumn-shed leaves may have a bearing on their survival in environments organically polluted by man.

STUDY AREA

Mont St. Hilaire (Fig. 1) is one of the Monteregian Hills, which lie in the plain of the St. Lawrence River valley between Montreal and the Sutton Mountains in southern Quebec. West Creek rises from small springs at an altitude of about 300 m and flows through thick beech–maple forest into Lac Hertel, which lies in the centre of the bowl-shaped mountain.

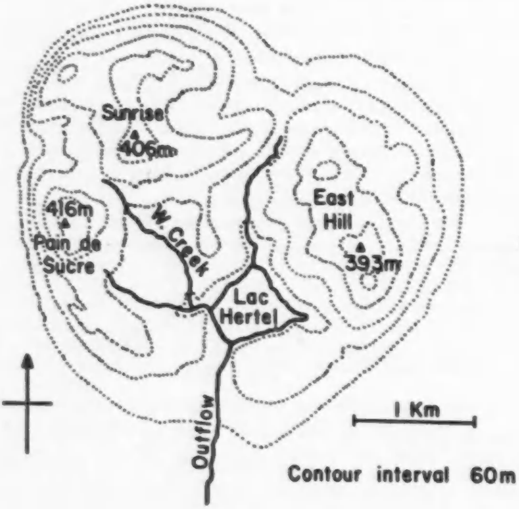

FIG. 1. The principal streams on Mont St. Hilaire, Quebec.

West Creek is 1.7 km long, but during most summers the upper third is dry. The width of the stream varies from 30 cm to 2 m, though some parts of the lower reaches flood to as much as 4 m during the April thaw. The water is cool and clear. Flow ranges from about 1 liter/sec in autumn after a dry summer to about 130 liters/sec in April.

There are no higher plants growing in the stream; algal growth is sparse and moss is found only on the exposed surfaces of immovable boulders. Leaves from the trees choke the stream during October and November and remain trapped behind fallen branches and stones for more than a year.

Four sampling stations were chosen, each being 60 m long. Station I was 300 m from the source (as established at the beginning of the study in November 1965). Here the width of the stream never exceeded 1.0 m and the water was only 2–5 cm deep over most of the station. The bottom consisted of sand, gravel, and pebbles with occasional boulders embedded in the gravel (substrate types classified according to Cummins (1962)). By July 25, 1966, the upper 40 m at station I were dry, leaving a few patches of damp gravel and leaves. Further downstream were some small isolated pools, 20–30 cm in diameter and 3–8 cm deep, which retained some water throughout the autumn.

Station II was immediately above a small pond that had been dug halfway downstream in 1964. The upper limit of station II coincided with the upper limit of flowing water during summer and autumn of 1966. The stream bed differed from that of station I only in having flat stretches of sand and silt at the lower end. The water seldom exceeded 8 cm in depth.

Station III was immediately below the pond. The upper 7 m of the station was a narrow stretch of gravel and pebbles over which the water flowed swiftly. Beyond, the water flowed sluggishly over sand and silt, 3–12 cm deep and 1–2 m wide. Three small tributaries, two of them temporary, entered the stream at this station. The third, an underground stream of seepage water, precipitated ferric hydroxide where it surfaced, about 2 m from the main stream. The dissolved oxygen concentration at the mouth of the tributary was less than 1 mg/liter.

Station IV was 300 m from where the stream entered Lac Hertel. At this station, the increased gradient of the valley floor allowed the water to flow more rapidly and the bottom consisted of boulders, cobbles, and pebbles resting on gravel with occasional sandy patches. The depth and width of the water were somewhat greater than at the higher stations but varied greatly with the seasons.

Mont St. Hilaire had a warm summer in 1966 and long cold spells in the preceding and following winters (Fig. 2). The mean air temperatures between December and March in 1965/66 and 1966/67 were −6.4 and −8.0 C respectively (P. D. Baird, personal communication).

The water temperature in West Creek ranges from a low of 0 C in January and February to a high of 17–18 C. Between January and March, ice forms at the sides of the stream, often developing into ice bridges on which snow accumulates. During 1966 and 1967 the difference in water temperature between points along the stream was small and stations I and IV reached the same

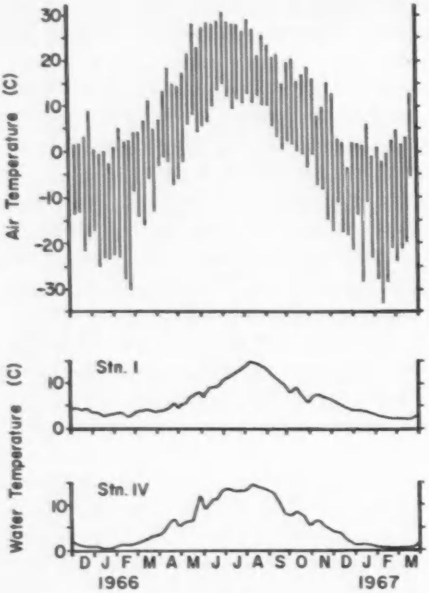

FIG. 2. Weekly records of maximum and minimum air temperatures, and mean water temperatures, in West Creek from December 1965 to March 1967.

maximum, 17.2 C (Table 1), in August 1966. However the water temperatures nearer the source increased more slowly between April and June, decreased more slowly during autumn and remained 1–3 degrees C higher than lower stations during most of the winter (Fig. 2). The greatest weekly range in temperature, 7–8 degrees C, occurred at station IV in May, October, and November. Maximum diurnal variation at station IV was 4.5 degrees C.

Total alkalinity in West Creek and concentration of dissolved salts were low (Table 1) reaching minima at the time of the spring thaw, and maxima at low water in September and October. The minimum *p*H (6.5) at station I was recorded in one of the small summer pools.

The minimum recorded concentration of dissolved oxygen and the lowest saturation level (Table 1) occurred in October, when the volume of water was low and fallen leaves choked the stream. Water samples from several points along the stream during the autumn showed that low oxygen concentrations persisted until late November, when temperatures were low, and increased flow began to thin out the leaves. Values of dissolved oxygen and saturation at all stations were highest between December and April, the levels dropping slightly when ice covered the stream.

A near maximum turbidity of the water at station IV on April 1, 1967, when the snow was melting, was 24 ppm SiO_2. The colour of the water was always less than 3 Pt colour units.

TABLE 1. Physical and chemical analyses of water samples from West Creek between November 1965 and April 1967.

	Station I		Station IV	
	Min	Max	Min	Max
Temp (*C*)	0	17.2	0	17.2
*p*H	6.5	7.6	7.1	7.4
Alkalinity (*mg* $CaCO_3$*/liter*)	7.0	15.0	16.2	35.0
Conductivity (*μmhos at 25 C*)	80	114	88	137
Dissolved oxygen (*mg/liter*)	5.7	12.2	7.8	13.4
% saturation oxygen	48.5	95.5	66.0	100.0

METHODS

The stream insects were studied from November 1965 until March 1967, but a regular sampling programme was not begun until March 1966. Monthly samples of the fauna were collected at each station. To observe changes in the stream as a whole at frequent intervals, station I was sampled in the last week of each month, stations II and III on 1 day near the middle of the month, and station IV during the 1st week of the month. In Tables 2–4, showing linear distributions of species, the total number collected of each species was corrected to a total per 30 samples per station so that relative abundances at each station are shown.

The stations were each divided into 12 substations 5 m long. To avoid denuding the subtrate in any one area and to ensure that each sample was of the undisturbed fauna, a different substation was sampled each month. Within the substation, samples were taken from all habitat types that lay on a diagonal transect. The habitat types sampled were sand, gravel, stones (cobbles and pebbles), leaves, and detritus. The term detritus was given to finely divided leaf material lying on pool bottoms, or near the stream margins. The leaf habitat consisted of fallen beech, maple, and other hardwood leaves that were loosely piled in slow flowing areas, or were trapped behind stones or fallen branches. During sampling, mixed substrates, such as leaves with stones or stones with sand, were purposely avoided; but silt concealed between leaves or stones was inevitably collected in many samples. Two to five samples were taken per station per month, depending on the number of habitat types encountered per transect. Conditions allowing the accumulation of detritus prevailed mainly in the late summer, so that samples of detritus were collected only from June to October in 1966, and in January and March in 1967. Sand at stations II, III, and IV was also sampled irregularly because sandy areas were not found in all transects. No quantitative samples were taken from sand at station I.

A Hess sampler enclosing 0.07 m^2 was used on sand and gravel, the substrate being disturbed to a depth of about 10 cm. Where the stream bed was uneven, an open half-cylinder was worked into the bottom more easily and placed on the downstream side of stones or twigs with the lateral wings of the frame curved round them. A semicircular brass rod was clipped on the half-cylinder to mark the anterior border of the area to be sampled (0.07 m^2). Both samplers were covered with Nitex net having a mesh size of 452 μ (16 threads/cm).

The importance of the mesh size used for collecting-nets and sieves has been emphasized by Jonasson (1955), Macan (1958), Hynes (1961), and Maitland (1966). A net of 16 threads/cm was chosen as being fine enough to retain all but the smallest insects, yet allowing the ubiquitous silt and leaf detritus that would clog a finer net to be washed away.

All animals and materials retained by the net were transferred to pint-sized plastic pots after rough sorting to remove large carnivores. The pots were anchored in the stream to maintain the organisms at normal water temperature while other samples were collected. The time taken to transport the organisms from the stream to a cool laboratory (12 C) was 1–1½ hours. The insects

TABLE 2. Linear distributions of Ephemeroptera, Plecoptera, and Trichoptera in West Creek between March 1966 and March 1967 on basis of numbers of specimens per 30 samples (the numbers of samples taken are given in parentheses). +, <10 specimens; ++, 10–100; +++, 101–200; ++++, >200.

	Station			
Species	I (32)	II (30)	III (28)	IV (50)
Ephemeroptera				
Siphlonurus sp.	+		+	
Ameletus "C"	++	+++	++++	++
Ameletus "B"	++	+++	+	++
Paraleptophlebia sp.	++	++	++++	++
Ameletus "A"		++		+++
Ephemerella funeralis McDunnough		+	++	++
E. dorothea Needham		+	+	++
Iron sp.		+		++
Heptagenia sp.				++
Plecoptera				
Ostrocerca albidipennis Walker	++++	++++	++++	
Leuctra spp.[a]	++	++	++	++
Alloperla onkos Ricker	++	+++	++	++
Soyedina vallicularia Wu	+	++	++	++
Isoperla similis (Hagen)	+	+	++	++
Allocapnia nivicola (Fitch)		++++	++	++
Amphinemura wui Claassen		++	+	++
Isoperla holochlora (Klapalek)			+	++
Allonarcys proteus Newman				++
Trichoptera				
Pseudostenophylax uniformis (Betten)	++	+		
Lepidostoma "C"	+++	+		
Neophylax aniqua Ross	++	++		
Ironoquia lyrata (Ross)	+	+	+	
Rhyacophila vibox Milne	++	+++	++	++
Lepidostoma "B"	++++	++++	++	++++
Lepidostoma "A"	++	++	++	+
Pycnopsyche gentilis (McLachlan)	+	++	++	++
Parapsyche apicalis (Banks)	+	++	++	+++
Pycnopsyche luculenta (Betten)		++	++	++
Polycentropus sp.		++	++	+
Rhyacophila minora Banks		++	+	++
Psilotreta sp.		+	+++	+
Molanna blenda Sibley		+	++	+
Pycnopsyche scabripennis (Rambur)		+	+	++
Phylocentropus sp.			++	
Oligostomis pardalis Walker			+	
Glossosoma sp.			+	++
Neophylax ornatus Banks				+
Wormaldia sp.				++
Neophylax nacatus Denning				+++

[a] *Leuctra tenella* Provancher and *L. truncata* Claassen.

TABLE 3. Linear distributions of Coleoptera, Neuroptera, and Diptera (excluding Chironomidae) in West Creek between March 1966 and March 1967 on basis of numbers of specimens per 30 samples (the numbers of samples taken are given in parentheses). +, <10 specimens; ++, 10–100; +++, 101–200; ++++, >200.

Species	Station			
	I (32)	II (30)	III (28)	IV (50)
Coleoptera				
Cymbiodonta blanchardi Horn	++	+	++	+
Agabus ambiguus (Say)	+	+	++	+
Helichus striatus LeConte	+		+	+
Hydrobius melaenum Germar		+	++	+
Promoresia sp.				++
Neuroptera				
Sialis sp.		++	++	+
Diptera				
Tipulidae[a]				
Pedicia albivitta Walker	++	+		
Dicranota sp.	+++	+++	++	++
Molophilus hirtipennis (O.S.)	+	+++	++	+
Tipula fraterna Loew	+	++	++	+
Hexatoma spinosa (Loew)	+	++	+	+
Tricyphona sp.	+	+	+	
Ormosia sp.		++	++	
Tipula strepens Loew		+		
Pilaris sp.		+	+	
Tipula abdominalis (Say)		++	++	+
Geranomyia sp.		+	+	+
Pseudolimnophila sp.		+		+
Limnophila sp.		++	++	++
Tipula "A"				++
Ptychopteridae				
Ptychoptera quadrifasciata Say		+	++	
Bittacomorpha clavipes (Fabricius)			++	
Simuliidae				
Twinnia tibblesi Stone and Jamnback	++++			
Simulium aureum (Fries)	+	+		
S. latipes (Meigen)	++	+	++	+
Prosimulium mixtum Syme and Davis	++++	++++	++++	++++
Cnephia mutata (Malloch)	++	+++	+++	++
Simulium tuberosum (Lundström)		+		+
Dixidae				
Dixa modesta Johannsen	++	+	+	++
Psychodidae				
Pericoma sp.	+			+
Ceratopogonidae				
Bezzia sp.	+	+++	+++	++
Empidae				
Roederiodes sp.	+	++	++	++
Hemerodromia sp.	+			+

[a] Ten other tipulid species were identified to tribe only.

TABLE 4. Linear distributions of Chironomidae (Diptera) in West Creek between March 1966 and March 1967 on basis of numbers of specimens per 30 samples (the numbers of samples taken are given in parentheses). +, <10 specimens; ++, 10–100; +++, 101–200; ++++, >200.

Species	Station			
	I (32)	II (30)	III (28)	IV (50)
Tanypodinae				
Conchapelopia sp.	+++	+++	++	++
Zavrelimyia sp.	++			++
Trissopelopia sp.	+	+++	++++	+
Psectrotanypus sp.		++	+	++
Macropelopia sp.		++	++	+
Paramerina sp.		+	+	
Natarsia sp.			+	
Nilotanypus sp.				+
Chironominae				
Constempellina sp.	+			
Tripodura sp.	+			
Micropsectra spp.	++++	++++	++++	+++
Tanytarsus sp.	++	++	++	+++
Polypedilum (s.s.) sp.			++	
Chironomus (s.s.) sp.			+	
Orthocladiinae				
Parochlus kieffeii (Garrett)	++++	+		
Sympotthastia fulva (Johannsen)	+++	+		
Pseudodiamesa pertinax (Garrett)	++++	+	++	
Diamesa spp.[a]	+++	++	+	++
Prodiamesa sp.	++	+	++	
Rheocricotopus sp.	++++	++	+	+
Heterotrissocladius sp.	+++	+++	++	+
Brillia sera Roback	++++	++	++	++
Diplocladius sp.	++	++	+	+
Nanocladius "C"	+++		+	++
Nanocladius "A"	++	+		++
Trissocladius sp.			+	+
Nanocladius "B"				+
Chaetocladius sp.				+
Cardiocladius sp.				+
Corynoneura group[b]	++	++	++	++
Metriocnemus group[c]	+++	++++	++	++

[a] Includes *D. nivoriunda* (Fitch).
[b] Includes *C. taris* Roback and *Thienemanniella* sp.
[c] Includes *Parametriocnemus* sp., *Paraphaenocladius* sp., and *Pseudorthocladius* sp.

were kept alive as long as possible because the presence of leaf detritus made sorting by flotation impossible. Whole leaves were rinsed and removed individually from the containers and other materials were washed many times in cold water, which was poured off through a net identical with those used in the field. Insects were removed by hand until no more were visible; then a few drops of concentrated formalin were added to the water in the sorting basin, causing any remaining concealed specimens to wriggle violently. Subsequent microscopic examination of samples of the remaining bottom materials showed that in most instances less than 1% of the organisms were overlooked. During June, July, and August, all sediments were checked microscopically to remove chironomid larvae.

All specimens except blackfly larvae were placed temporarily in 5% formalin to preserve the natural colour patterns as an aid to identification. Blackfly larvae were killed and preserved in 95% ethanol. After the insects had been identified, measured, and counted, they were preserved in 70% ethanol. Subsequently, representative specimens of the age-classes of each species were weighed so that the biomass could be calculated for each habitat and for the stream as a whole (Mackay and Kalff, 1969).

The most reliable measurement used to group immature insects into age-classes was the width of the head (including compound eyes). The length of the lateral border of the mesonotum was also measured on nymphs of Ephemeroptera and Plecoptera. Measurement of length alone was unreliable in most instances because of shrinking or telescoping of the abdomen. Length was used as the distinguishing character for the dipterous families Tipulidae, Ceratopogonidae, and Empidae as the head capsules in these groups were incomplete or inverted, or too narrow for changes in size to be measured accurately. However, in all immature insects increase in length during the year gave a good indication of growth rate, and changes in total length were more gradual than changes in head width and mesonotum length. Therefore approximate total lengths are included in the figures showing larval life histories.

The average monthly abundance of common insects in each of the five habitats was based on samples from four stations (with the exception of sand at station I). The species considered as common were those whose numbers exceeded 50/m^2 during at least 1 month; thus common species having only a short aquatic season were not excluded. The arbitrary value chosen to distinguish common from occasional species proved satisfactory in that all species that were collected consistently in small numbers fell into the common category.

SAND FAUNA

Common species of insects living in or on sand (Fig. 3) were members of the Trichoptera (2 spp.) and Diptera (17 spp.). Larvae of the caddisfly *Psilotreta* sp. were taken from the surface of bare sand, usually from midstream areas. A few collections contained nymphs of Ephemeroptera and Plecoptera, most of which were more common in other habitats. However, the mayfly *Siphlonurus* sp. was found only swimming in open water above bare sand. *Molanna blenda* (Trichoptera) was another occasional member of the sand fauna, but was rarely collected from bare sand; most specimens were found in areas where thick piles of leaves had been removed before the sand below was disturbed.

The most numerous larvae covered by sand were Tipulidae; this family was also represented by the most common species (7). Other common Diptera were Ptychopteridae (2 spp.), Chironomidae (6 spp.), Ceratopogonidae (1 sp.), and Empidae (1 sp.)

The average monthly abundance of the common sand fauna species (Fig. 3) shows that October and February have similar features. The data for October 1966 are the results of a single sample of sand from the mouth of the nearly deoxygenated tributary at station III. The sand was mixed with ferric hydroxide and was ochrous in colour. Three sand samples were collected in February 1967, including another ochrous sample taken immediately downstream of the tributary. All species common only in October and February were members of the ochrous sand fauna.

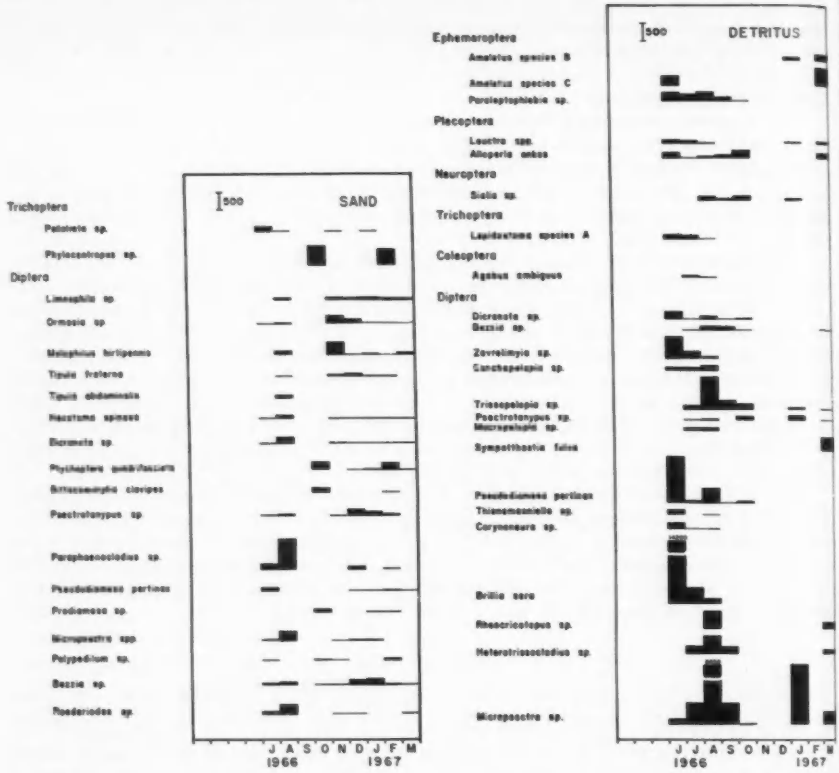

FIG. 3. The average numbers/m² per month of common insects in the sand habitat, West Creek, between July 1966 and March 1967, and in the detritus habitat between June and October 1966 and in January and March 1967.

Phylocentropus sp. larvae built their partially buried sand tube systems near the stream margins, and appeared to tolerate very low concentrations of dissolved oxygen.

None of the sand fauna species were collected regularly enough to provide reliable life history information.

DETRITUS FAUNA

Samples of detritus were taken from areas of slow-flowing water where detritus lay over sand, or occasionally over gravel. The detritus fauna, like some members of the sand fauna, was often found in poorly oxygenated water; between July and October the dissolved oxygen concentration was sometimes reduced to less than 50% of saturation (Table 1). The detritus fauna also resembled the sand fauna in that most of the 23 common species were Diptera (Fig. 3). However, in detritus the Chironomidae was the predominant family and species of *Micropsectra* were abundant at all seasons. The fauna included species in five other insect orders, but only *Paraleptophlebia* sp. (Ephemeroptera) and *Sialis* sp. (Neuroptera) were more common in detritus than in other habitats.

The data for June 1966 are the results of a single sample at station I, and the high numbers of *Brillia sera* and *Pseudodiamesa pertinax* were peculiar to this station. The results for July and August are based on samples from all stations and therefore are representative of West Creek as a whole.

GRAVEL FAUNA

Insect species diversity was notably greater in the gravel habitat than in sand; 27 species were common in gravel, representing five orders (Fig. 4). Some species such as *Cymbiodonta blanchardi* and *Promoresia* sp. (Coleoptera), and *Trissocladius* (Chironomidae) were apparently confined to the gravel habitat. Most of the remaining common species were found in other habitats also, either having a broad range in habitat preference or spending only part of their larval development in gravel.

Species appearing to have a broad range in substrate preference include the three species of *Ameletus* (Ephemeroptera) which were common in all habitats except sand, where they were found occasionally. *Alloperla oikos* (Plecoptera) was also common in all habitats except sand. Blackfly larvae, common in gravel, stones, and leaf habitats, were very abundant in gravel during winter and early spring. Between January and April, gravel areas at stations II and III immediately below the pond appeared black owing to large numbers of *Prosimulium mixtum* and *Cnephia mutata* larvae.

The three congeneric species of Ephemeroptera did not develop simultaneously, so that any overlap in seasonal occurrence of the species rarely involved nymphs of the same size (Fig. 5). *Ameletus* A hatched in August and grew continuously during the winter to emerge as the adult in March and April. *Ameletus* B nymphs first appeared during October or November, grew very slowly until January, and developed rapidly between February and April to emerge in May and June. The third species, *Ameletus* C, hatched in March and completed its development in 5 months.

Species that are members of the gravel fauna for a limited period include the stoneflies *Ostrocerca albidipennis*, *Isoperla similis*, and *Allocapnia nivicola*. Each of these species was common in gravel (Fig. 4) during months when the nymphs were small (Fig. 6–8). Young larvae of *Parapsyche apicalis*, collected between July and November (Fig. 9) were common on gravel during this period although previous records of the species have not noted this (Flint, 1961). Young larvae of the tipulid *Dicronota* sp. were also found in gravel but later instars were taken mainly from leaves trapped in small waterfalls.

Larvae of the chironomids *Sympotthastia fulva* and *P. pertinax* (Diamesini) were sometimes collected in large numbers from gravel, but only when this was mixed with organic detritus. The association between these two species and detritus is emphasized by their abundance in the pure detritus habitat

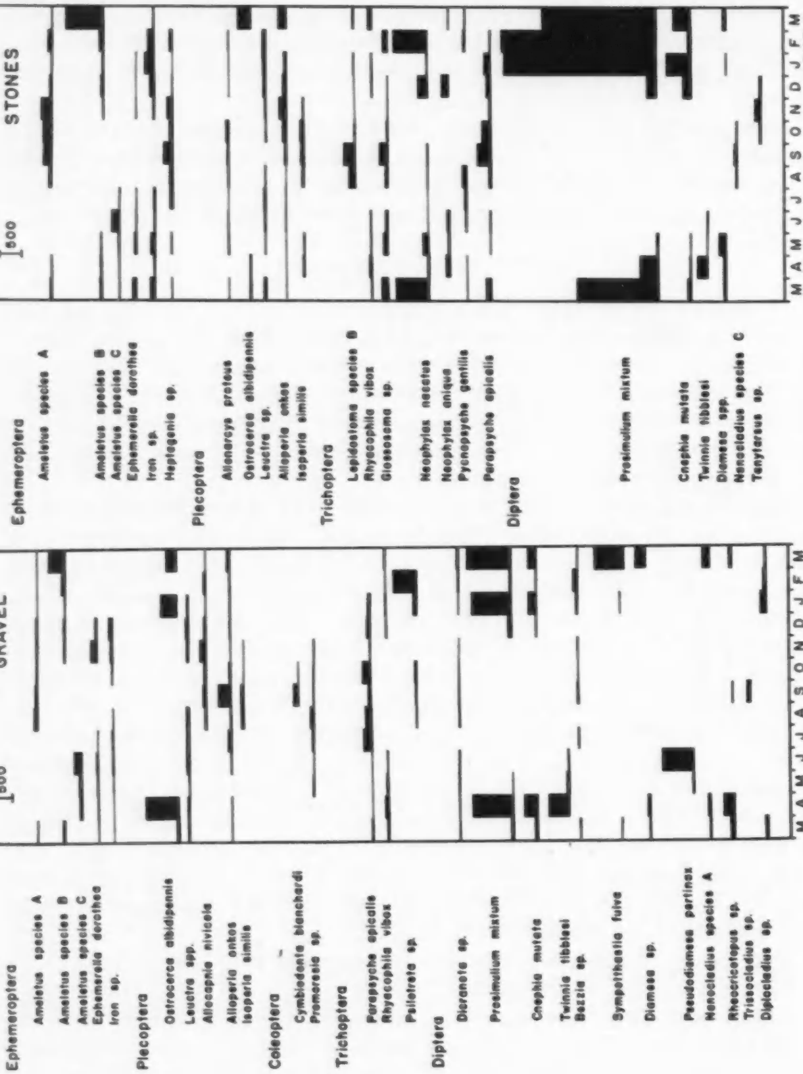

FIG. 4. The average numbers/m² per month of common insects in the gravel habitat and in the stone habitat. West

(Fig. 3). In both detritus and gravel the two species were clearly separated temporally. Larvae of *S. fulva* were collected between January and March, adults between February and April; larvae of *P. pertinax* were collected from May through August, pupae and adults during September and October.

When station I was dry between July and October 1966, samples of damp gravel below the surface of the stream bed revealed larvae of many species,

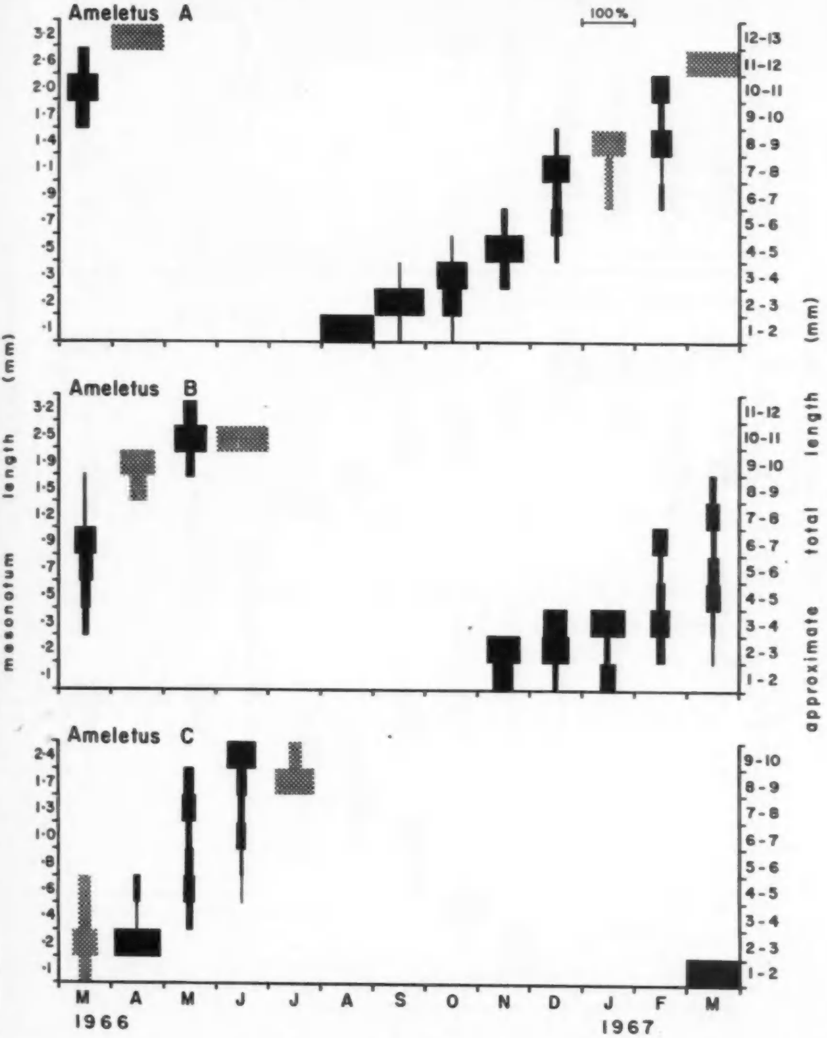

FIG. 5. Growth of nymphs of three species of *Ameletus* (mayflies), West Creek, 1966–67. (Each histogram shows percentage of total specimens per month having size indicated. Cross-hatched histograms based on <10 specimens/month.)

including the caddisflies *Pseudostenophylax uniformis* and *Lepidostoma* "C," which must have burrowed into the substrate as the water receded. Other species collected from damp gravel and sand at this station included *Pseudorthocladius* sp., *Paraphaenocladius* sp., and *Brillia sera* (Chironomidae); and young larvae of *Molophilus hirsutipennis* and *Dicranota* sp. (Tipulidae).

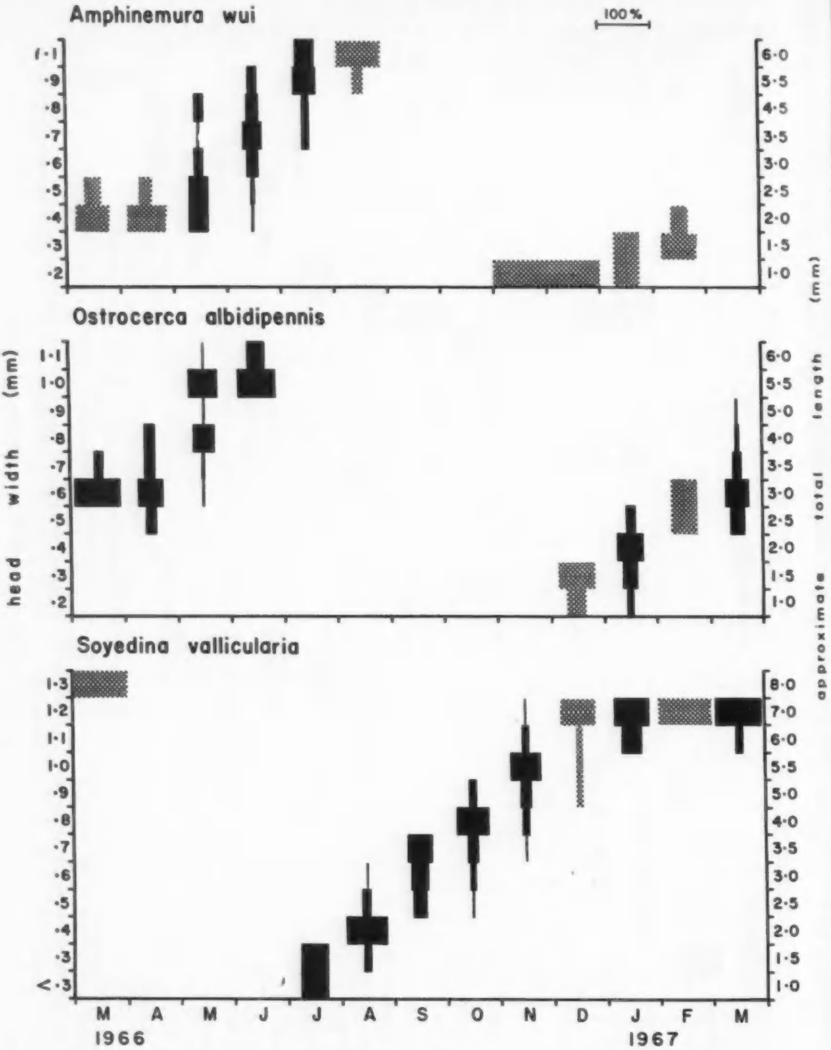

FIG. 6. Growth of nymphs of the nemourid stoneflies *Amphinemura wui*, *Ostrocerca albidipennis*, and *Soyedina vallicularia*, West Creek, 1966–67. (Each histogram shows percentage of total specimens per month having size indicated. Cross-hatched histograms based on <10 specimens/month.)

STONE FAUNA

The number of common species in the stone fauna (Fig. 4) was slightly less than that in gravel, but included insect larvae found only on the surfaces of stones. These were the mayfly *Heptagenia* sp., and the caddisflies *Glossosoma* sp., *Neophylax nacatus*, and *N. aniqua*. A third species of *Neophylax*, *N. ornatus*,

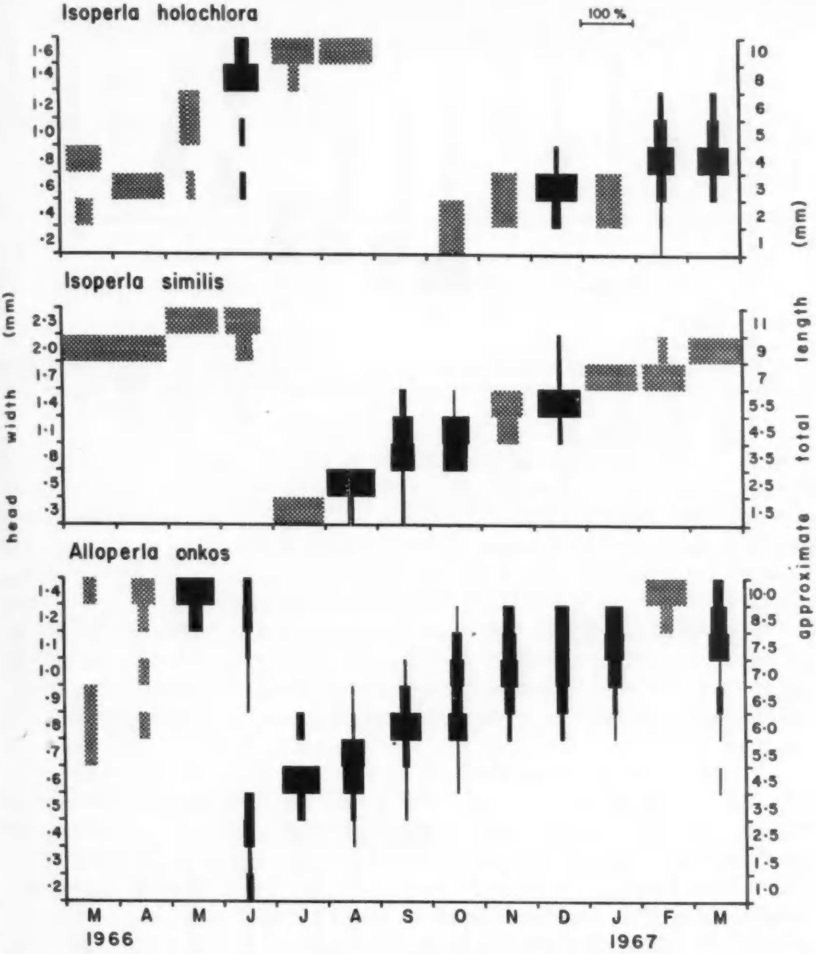

FIG. 7. Growth of nymphs of the stoneflies *Isoperla holochlora*, *I. similis*, and *Alloperla onkos*, West Creek, 1966–67. (Each histogram shows percentage of total specimens per month having size indicated. Cross-hatched histograms based on <10 specimens/month.)

was collected occasionally. *Neophylax nacatus* was the commonest of the three species.

The Ephemeroptera in general, and especially *Ameletus* species A and B, were more abundant among stones than they were in other habitats. *Heptagenia* sp. and *Iron* sp. nymphs were found clinging to the lateral and ventral surfaces of stones. The Plecoptera common on stones included the large *Allonarcys proteus* which has a 2–3 year life cycle (Fig. 10). Most of the *A. proteus* nymphs in the stone fauna were in their late 2nd or 3rd year of

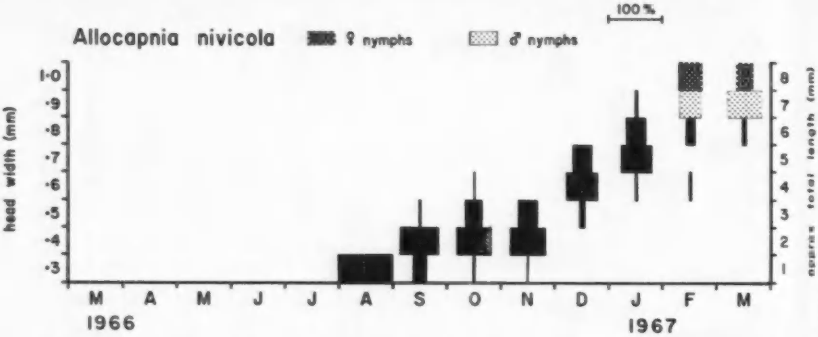

FIG. 8. Growth and sexual dimorphism in nymphs of *Allocapnia nivicola*, West Creek, 1966–67. (Each histogram shows percentage of total specimens per month having size indicated.)

growth, for the earlier instars were usually in the leaf habitat. Several caddisfly species, including *Rhyacophila vibox* and *Pycnopsyche gentilis*, moved into the stones during their terminal larval instar and pupated on the ventral surfaces of stones. Larvae of *P. apicalis*, mainly medium and large sizes, were collected from stones throughout the year, the long hatching period in gravel allowing recruitment into the stone fauna from August through March.

The Simuliidae were the most numerous insect larvae in winter and spring; they clung to the upper surfaces of stones, usually on the downstream edges. *Prosimulium mixtum* and *C. mutata* were the predominant larvae during winter, being followed in smaller numbers in spring by *Twinnia tibblesi* (Fig. 11).

Young larvae of the caddisfly *N. nacatus* were first collected in December (Fig. 9); they grew during winter and spring until all were at the prepupal stage by the end of June. Pupae were found up to September but adults were not collected. *Neophylax aniqua* appeared to have a similar life history, but, as described below, it was not found in the same parts of West Creek as *N. nacatus*. *Neophylax ornatus* differed in its life cycle, as young larvae were collected in August, mature larvae in December.

The majority of larvae of the caddisfly *P. apicalis* appeared to complete their development in one year (Fig. 9). But although no terminal-instar larvae were collected in August 1966, their appearance in September suggests that some individuals may spend a second winter in the stream before emerging as adults.

LEAF FAUNA

In all areas of West Creek, leaves supported the greatest number of species. There were 43 common species in 5 orders, more than half of the species being Diptera (Fig. 12). The commonest larvae in the habitat were those of a small caddisfly, *Lepidostoma* "B," highest numbers/m^2 being collected in

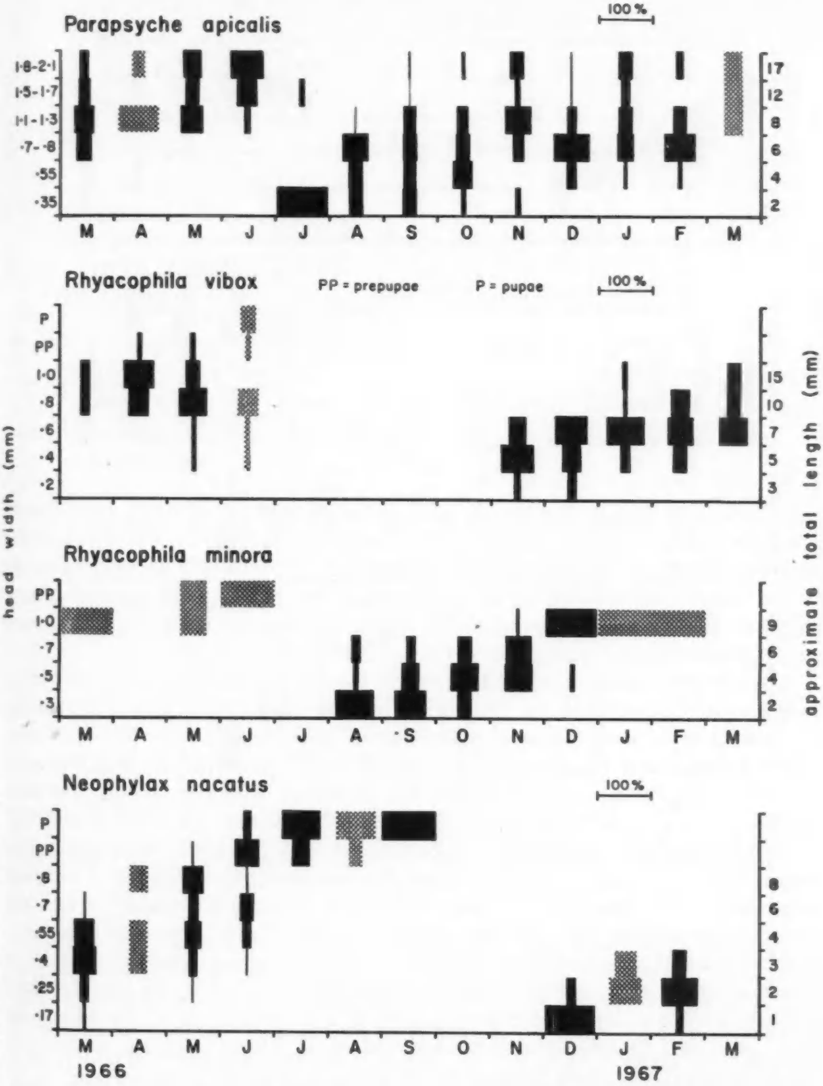

FIG. 9. Growth of immature stages of four species of caddisflies, West Creek, 1966–67. (Each histogram shows percentage of total specimens per month having size indicated. Cross-hatched histograms based on <10 specimens/month.)

September. Nymphs of the plecopteran *O. albidipennis* which were found mainly in gravel during winter, increased in numbers in the leaf habitat in spring. Blackfly larvae were found on leaves caught on branches and stones in the current; *P. mixtum* was the predominant species between December

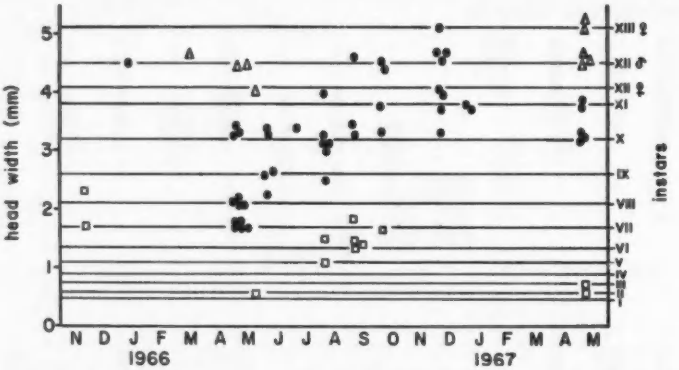

FIG. 10. Head widths of *Allonarcys proteus* nymphs taken from station IV, West Creek, in relation to known instars reared by Holdsworth (1941). □, 1st year of growth; ●, 2nd year; △, 3rd year. Each symbol represents one specimen.

and April. The tipulid *Tipula* "A" and the psychodid *Pericoma* sp. were found in closely packed leaves near the water surface. The mayfly *Paraleptophlebia* sp., the stonefly *A. nivicola,* and the chironomid *Trissopelopia* sp. were in leaves resting loosely in slow-flowing areas. Several chironomid species were abundant for short periods and the numbers of maturing larvae of the tipulid *Dicranota* sp. increased during spring and early summer.

Wherever a genus was represented by more than one species (or subgenera in the case of *Amphinemura, Ostrocerca,* and *Soyedina* in the genus *Nemoura*), the species differed in the time of their larval growth periods or in their linear distributions along the stream. In most instances there was a clear temporal succession of species, as shown by the common Ephemeroptera, *Ameletus* species A, B, and C being followed by *Paraleptophlebia* sp. (Fig. 5 and 12).

The nemourid stoneflies *O. albidipennis* and *Soyedina vallicularia* also had different periods of nymphal growth and emergence (Fig. 6). *Soyedina vallicularia* developed continuously from July through December, when the nymph was almost fully grown, then remained about the same size until March when it moulted once or twice before emerging. Young nymphs of *O. albidipennis* appeared in December, grew during the winter and emerged in May and June. A third common nemourid in the leaf habitat, *Amphinemura wui,* resembled *O. albidipennis* in size and in its life cycle (Fig. 6) but was rarely found in the same part of the stream.

Two species of *Isoperla* (stoneflies) occurred in the leaf habitat although *Isoperla holochlora* was collected only occasionally. *Isoperla similis* developed slowly but continuously from July to the following June, whereas small nymphs of *I. holochlora* appeared in October, grew little during autumn and winter, but completed their development rapidly the following summer (Fig. 7).

A similar temporal separation existed between the caddisflies *R. vibox* and *R. minora* (Fig. 9). Young larvae of *R. minora* were first collected at the

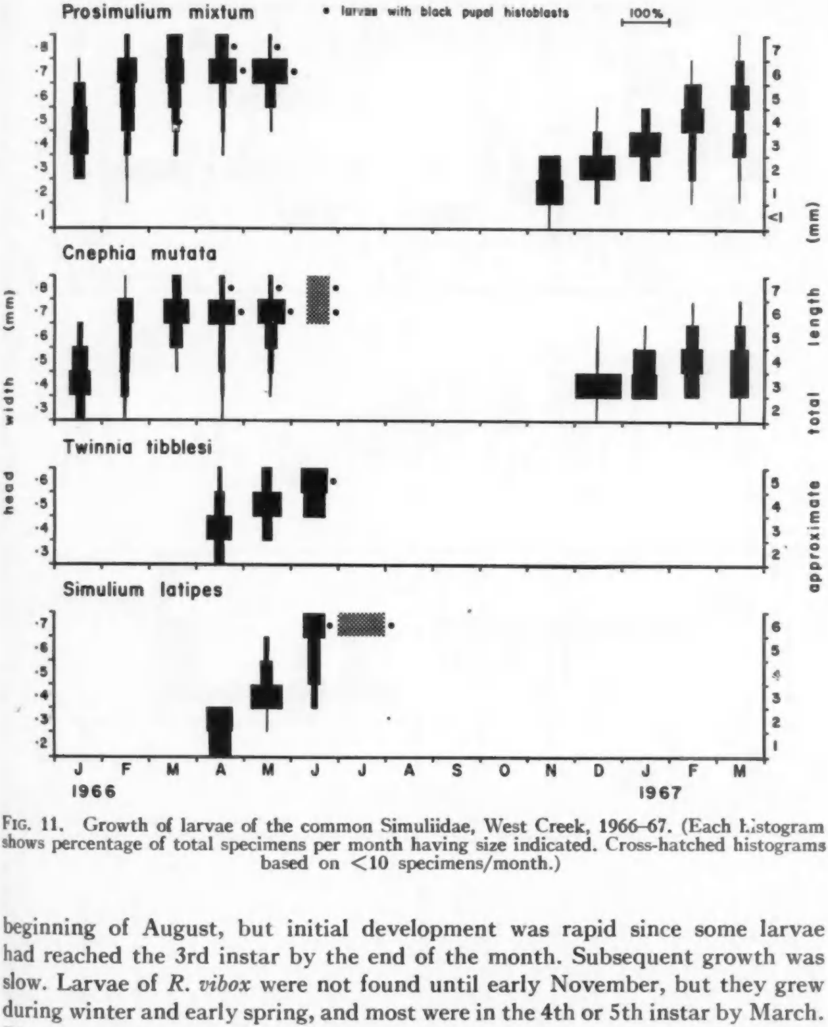

FIG. 11. Growth of larvae of the common Simuliidae, West Creek, 1966–67. (Each histogram shows percentage of total specimens per month having size indicated. Cross-hatched histograms based on <10 specimens/month.)

beginning of August, but initial development was rapid since some larvae had reached the 3rd instar by the end of the month. Subsequent growth was slow. Larvae of *R. vibox* were not found until early November, but they grew during winter and early spring, and most were in the 4th or 5th instar by March. The younger larvae collected in May and June, though attributed to *R. vibox*, may have been those of another species; but none were reared, and older stages were not found in later months.

The three species of *Lepidostoma* (caddisflies) that occurred in the leaf habitat also showed temporal separation. *Lepidostoma* A larvae appeared to hatch in December (Fig. 13) but little growth took place before March. During the summer months *Lepidostoma* A grew rapidly and emerged in August. Adults of species B and C emerged early in the summer, but, unlike species A, young larvae appeared from the eggs immediately. *Lepidostoma* B larvae

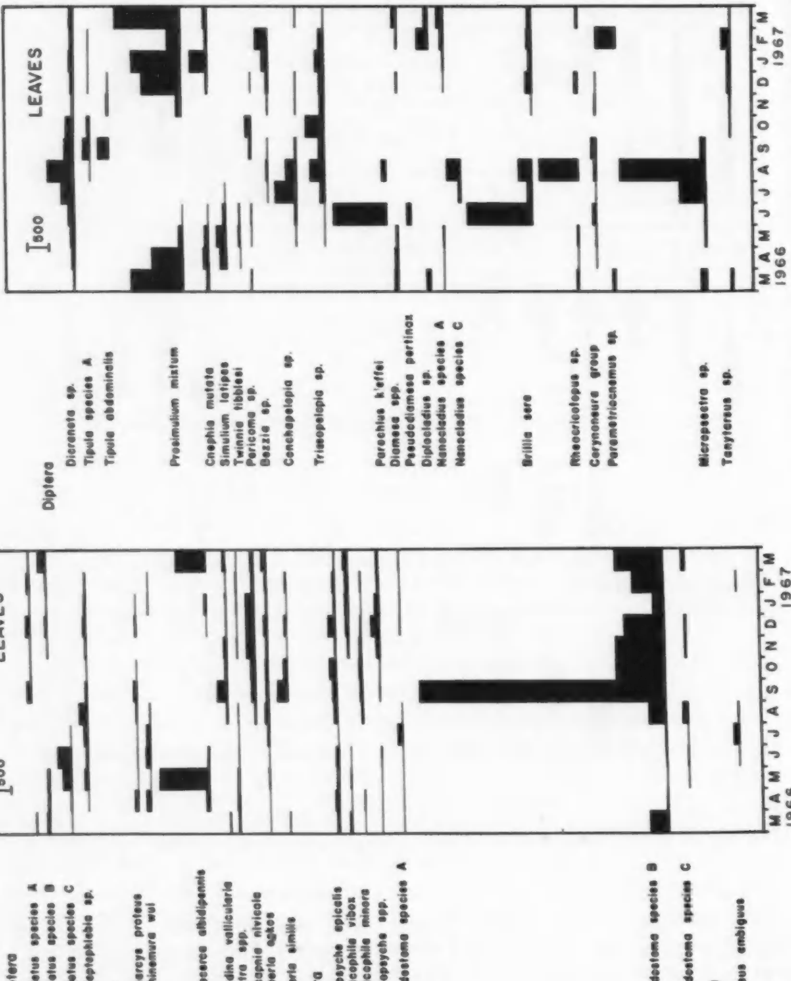

FIG. 12. The average numbers/m² per month of common insects in the leaf habitat, West Creek, between March 1966 and March 1967.

grew steadily throughout the summer and autumn and all were fully grown by the end of the following March. However, the majority of *Lepidostoma* C larvae completed their development in 3 months and remained the same size during the winter. As well as having specific life cycle patterns, the three species differed in their spatial distribution in the leaf habitat. *Lepidostoma*

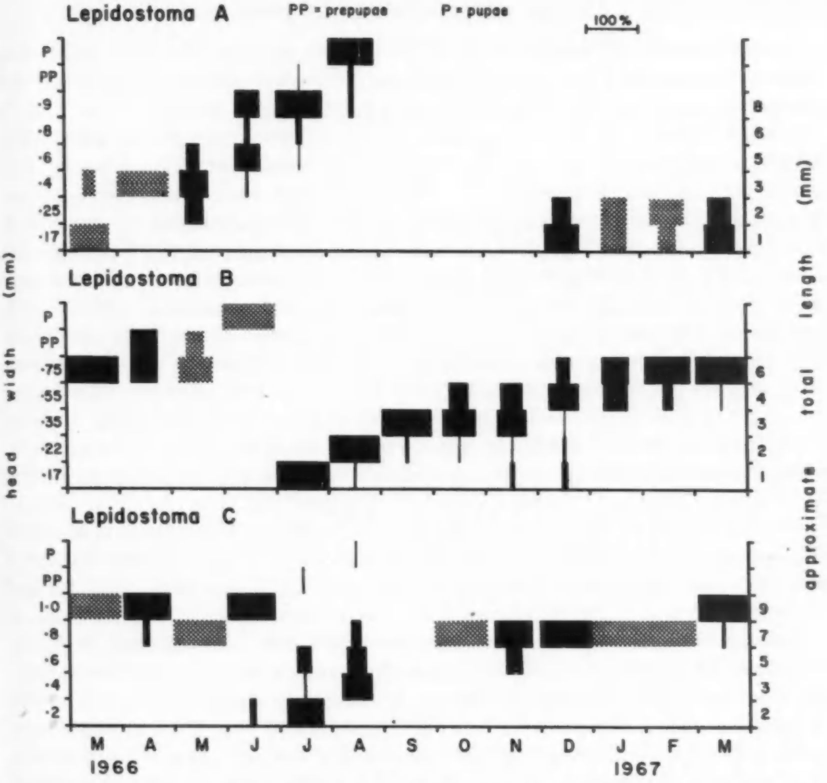

FIG. 13. Growth of larvae of three species of *Lepidostoma* (caddisflies), West Creek, 1966–67. (Each histogram shows percentage of total specimens per month having size indicated. Cross-hatched histograms based on <10 specimens/month.)

A was found mainly on leaves in pools and in slow-moving water, whereas *Lepidostoma* B was most common on leaves trapped on fallen branches in the current but was collected from all types of leaf habitat. *Lepidostoma* C was restricted to leaves near the source of the stream.

The seasonal relationships between species of Diptera were traced in detail only for the Simuliidae, whose larvae could be identified at all stages of development. The succession of species in the leaf habitat was similar to that in the stone habitat, but a second common spring species, *Simulium latipes* (Fig. 11) was present on leaves. Although the remaining common species of Diptera in the leaf habitat (Fig. 12) were less easy to recognise, *Nanocladius* "A" clearly alternated with *Nanocladius* "C," and high numbers of *Conchapelopia* sp. larvae in July were followed by high numbers of *Trissopelopia* sp. in October.

LINEAR DISTRIBUTIONS OF INSECTS

Very few of the insects in West Creek were equally abundant at all four collecting stations. The number of species per station tended to increase from station I to station IV in most insect groups (Tables 2–4).

Eight species of Ephemeroptera (Table 2) were collected at station IV, including *Heptagenia* sp. which was confined to this station, whereas only 4 species were found at station I. *Ameletus* C and *Paraleptophlebia* sp. were the most abundant species at any one station, in both instances at station III.

The number of Plecoptera species (Table 2) increased from 5 or 6 species at station I to 8 or 9 species at station IV. *Allonarcys proteus* was found only at station IV, and *I. holochlora* was found only at stations III and IV. The remaining 8 species of Plecoptera were collected from at least three stations. *Ostrocerca albidipennis* was very abundant at the upper stations but absent from station IV; even at station III, nymphs were found only on one occasion in May in a mass of leaves caught on some twigs just below the pond, and had probably been washed down by high water from station II. However, *A. wui*, resembling *O. albidipennis* in size and life history, was more common at station IV than at the other stations.

Nine species of Trichoptera (Table 2) were collected at station I, of which *P. uniformis*, *Lepidostoma* C, and *N. aniqua* were found only at stations I and II. *Ironoquia lyrata* was collected occasionally at stations I and II, and one specimen was found at station III in the mouth of a temporary tributary. However, *I. lyrata* was not found elsewhere at station III and had probably been washed in from the tributary, where the larvae were common. The remaining five species of Trichoptera collected at station I were more or less evenly distributed along the stream. Station II supported six species more than station I, all six occurring also at stations III and IV. Three more species were collected at station III, of which *Phylocentropus* sp. and *Oligostomis pardalis* were confined to this station. Except for the last two species and *I. lyrata*, all Trichoptera found at station III were found also at station IV. In addition, there were three species peculiar to station IV: *N. nacatus*, *N. ornatus*, and *Wormaldia* sp.

Coleoptera, Neuroptera, and Tipulidae (Table 3) were all represented by fewer species at station I than at stations II–IV. Members of the Tipulidae and Ptychopteridae were found chiefly at stations II and III. Exceptions were the leaf-dwelling tipulids: *Pedicia albivitta* mainly at station I, *Tipula* "A" only at station IV, and *Dicranota* sp. at all stations. The Simuliidae (Table 3) were represented by more species at stations I and II than at stations III and IV. *Twinnia tibblesi* was found only at station I, but was abundant there.

Of the Chironomidae (Table 4) only the subfamily Tanypodinae showed a marked downstream increase in the number of species. Station I was characterized by low numbers and few species of Tanypodinae and Chironominae, but had high numbers and many species of Podonominae and Orthocladiinae. At the other stations the Tanypodinae were more diverse and the numbers of Orthocladiinae declined.

DISCUSSION

The species composition of insects in West Creek is influenced considerably by the thick deciduous forest surrounding the stream, and by the climate. Only a small amount of light reaches the stream while the trees are in leaf, and during the winter the stream bottom is shaded by ice bridges and snow. The small amount of incident radiation appears primarily responsible for the near absence of autotrophic plants in West Creek. The food available to herbivores is almost entirely allochthonous organic material in the form of fallen leaves. The feeding habits of the insects were not studied beyond simple observations of behaviour, but information from other sources (Wu, 1923; Frison, 1929; Claassen, 1931; Chapman and Demory, 1963) shows that algal feeders in the stream were few, and that many of the common species feed on leaves or leaf detritus, e.g. *Paraleptophlebia* sp., *Allocapnia nivicola*, *Soyedina vallicularia*, *Allonarcys proteus*, and all species of *Lepidostoma*, *Pycnopsyche*, and the Simuliidae.

The cool water temperatures are maintained by the spring source and by the dense shade provided by the forest in summer. The amount of water that evaporates from the vegetation surrounding West Creek exceeds precipitation during summer (Baird, 1968), and as a result parts of the stream and its tributaries dry up. The drought not only has a direct effect on the fauna, but also allows the stream to be choked with fallen leaves in October when the water volume is lowest.

The distributions of the insect species are probably influenced chiefly by food and temperature; however, the size of the stream is also an important factor, because the smaller the stream, the more it is affected by environmental conditions and by the accumulation of leaves. Ross (1963) has related the distribution of many eastern Nearctic Trichoptera to the influence of deciduous forest, and the majority of the Trichoptera in West Creek are known to be cold water, small stream species (Ross, 1944, 1959; Flint, 1960, 1961, 1962; Wiggins, 1960). The three species of *Ameletus* (Ephemeroptera) in West Creek appear to be restricted to cool water. Neither *Ameletus* A nor *Ameletus* C was found in the Lac Hertel outflow stream (Fig. 1) where water temperatures in August exceeded 22 C, whereas both species occurred in West Creek (17 C) in August. *Ameletus* B, aquatic only during cool months (Fig. 5) was abundant in the outflow stream; since the nymphs complete their development and emerge as adults before the water temperature exceeds 10 C, they are able to avoid high summer temperatures. The Orthocladiinae (Chironomidae) are more characteristic of cool streams than warm streams, so the relatively high numbers of species and individuals in West Creek is in agreement with the generally cool temperatures. However, the abundance of *Pseudodiamesa pertinax* in small summer pools at station I was still surprising, as this species was previously known only from the Arctic and from high mountain streams in Utah and British Columbia (Oliver, 1959). All the Simuliidae of West Creek have been reported previously (Davies et al., 1962) as occurring in small streams, supporting the idea that a small stream has a characteristic fauna.

The various communities within West Creek are probably influenced greatly by the current velocity, which in turn determines substrate type, accumulation of allochthonous organic material, and dissolved oxygen concentration. The fauna of each substrate type (sand, gravel, and stones) is made up of organisms adapted to the particular niche size or surface area offered by the substrate particles, and to the speed of the current over the substrate. The burrowing members of the sand fauna are probably least affected by the size of the stream and accumulations of leaves since light and food materials in the habitat differ little from those for sand in other parts of the stream. However, the ability to tolerate low dissolved oxygen concentrations, as shown by the ochrous sand fauna, is advantageous in a stream that receives a large amount of slow-moving organic material every autumn. *Bittacomorpha clavipes* was confined to the mouth of the deoxygenated tributary at station III, but other species from this special habitat such as *Phylocentropus* sp. and *Ptychoptera quadrifasciata*, and also species in the detritus fauna, may be peculiar to West Creek and similar streams.

The insect community in gravel is the least specialised and includes many species more common in other habitats. However, the interstices of the substrate shelter early stages of Plecoptera, Trichoptera, and Diptera, especially during summer. The gravel presumably forms an important reservoir from which other habitats are stocked, and also protects many species from desiccation during periods of drought. The tendency of young larvae to move from gravel to leaves as they grow suggests that there may be insufficient food in the gravel habitat to support a large population of herbivores.

Stones in West Creek, having only sparse algal growth and no moss on them support fewer algal feeders and more leaf feeders than stony habitats described elsewhere (Sprules, 1947; Scott, 1958; Hynes, 1961). Since the current responsible for the stony substrate tends to flush fallen leaves from the stream bottom, the stones habitat probably suffers the same food shortage as gravel. The temporal separations of congeneric species of *Ameletus* and *Neophylax* (all assumed to be algal feeders) may be interpreted as examples of ecological segregation allowing the most efficient use of limited resources (Grant and Mackay, 1969). On the other hand, the high numbers of Simuliidae in the habitat are able to utilize the drifting organic detritus resulting from disintegration of fallen leaves.

The leaf and detritus faunas are the most important communities since they support the highest numbers of species and individuals per unit area of stream. Accumulations of leaves and detritus in the slow-flowing areas are typical of small streams in thick deciduous forest, yet the rich fauna has not been described before in terms of species associations. The fauna of fallen autumn leaves in October and November gradually changes the following spring to include species able to tolerate warmer temperatures and more detritus. Species living and feeding on entire leaves, e.g. *Lepidostoma* B, become more crowded as the leaves break down, resulting in very high numbers per unit area in September when few whole leaves remain. At this time the dissolved oxygen concentration in shallow pools is near the annual minimum and is further reduced by falling leaves. Yet both leaves and detritus continue to support a high number of species.

The linear distributions of the species (Tables 2–4) partly reflect the linear distributions of habitat types. The species peculiar to station III are members of the sand or detritus faunas typical of the slow-flowing areas at this station. Species confined to stony substrates are most abundant at station IV. However, the distinctive fauna at station I is more likely the result of summer drought, which restricts the species to those that can avoid desiccation. Some larvae, described above, appear to survive while buried in damp gravel. Others, as noted by Hynes (1958) for several species in Wales, have emerged as adults by the time the stream dries up. Either they lay drought-resistant eggs, or they have flight periods long enough to allow females to lay eggs when the stream flows again. The eggs of *Ostrocerca albidipennis* and members of the Simuliidae may tolerate desiccation, since the abundance of larvae (totalling more than 1000/m^2 in winter) at station I is unlikely to be due to chance, but adults were not observed later than August, when the stream was still dry. The eggs of *Lepidostoma* B may also be drought-resistant because a collection made at station I in November, when the water was just beginning to flow again, revealed many 1st instar larvae. This late hatching, some 4 months after the first appearance of the larvae at other stations (Fig. 13), may have been triggered by contact with water (G. B. Wiggins, personal communication).

The only insect whose distribution might have been affected by a temperature gradient in West Creek was the chironomid *Parochlus kieffeii* which was very abundant at station I in June 1966 when the water temperature was about 5 degrees C lower than at other stations. During the rest of the year the temperature gradient was not marked.

The presence of *Twinnia tibblesi* at station I only is explained by the behaviour of adult females of this species, which always fly to the upstream limit of flowing water before laying their eggs (D. M. Wood, personal communication). It is possible that the similar behaviour noted by Roos (1957) in some Trichoptera may account for the restriction of *Lepidostoma* C, *Pseudostenophylax uniformis*, and *Ironoquia lyrata* to stations I and II, as well as their ability to avoid desiccation.

The insect communities in West Creek together include a remarkably high number of species, their diversity evidently not being in proportion to the size of the stream nor reduced by the large quantity of organic matter. In each of the five habitats studied are many species apparently well adapted to the conditions found in a small cool stream flowing slowly through thick deciduous forest. The diversity of species is enhanced by the temporal or spatial segregation of congeneric species that allows many similarly adapted species to utilize the resources efficiently, yet reduces interspecific competition.

When more insect larvae have been identified to species level, it will be possible to compare the fauna of West Creek with that of similar streams.

If the insect communities described above are indeed characteristic of this type of stream, then the information on their distributions and seasonal occurrences in West Creek may be used in studies on water quality.

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REFERENCES

Baird, P. D. 1968. Mont St. Hilaire. McGill University Press, Montreal, Que. 24 p.

Chapman, D. W., and R. L. Demory. 1963. Seasonal changes in the food ingested by aquatic insect larvae and nymphs in two Oregon streams. Ecology 44: 140–146.

Claassen, P. W. 1931. Plecoptera nymphs of North America. Thomas Say Foundation, Baltimore, Md. 199 p.

Cummins, K. W. 1962. An evaluation of some techniques for the collection and analysis of benthic samples with special emphasis on lotic waters. Am. Midland Naturalist 67: 477–504.

Davies, D. M., B. V. Peterson, and D. M. Wood. 1962. The blackflies (Diptera: Simuliidae) of Ontario. Part I. Adult identification and distribution with descriptions of six new species. Proc. Entomol. Soc. Ont. 92: 70–154.

Flint, O. S. 1960. Taxonomy and biology of Nearctic limnephelid larvae (Trichoptera) with special reference to species in the eastern United States. Entomol. Am. 40: 1–120.

1961. The immature stages of the Arctopsychinae occurring in eastern North America (Trichoptera: Hydropsychidae). Ann. Entomol. Soc. Am. 54: 5–11.

1962. Larvae of the caddis fly genus *Rhyacophila* in eastern North America (Trichoptera: Rhyacophilidae). Proc. U.S. Natl. Museum 113: 465–493.

Frison, T. H. 1929. Fall and winter stoneflies or Plecoptera of Illinois. Ill. Nat. Hist. Surv. Bull. 18: 343–409.

Gaufin, A. R., and G. M. Tarzwell. 1956. Aquatic macroinvertebrate communities as indicators of organic pollution in Lytle Creek. Sewage Ind. Wastes 28: 906–924.

Grant, P. R., and R. J. Mackay. 1969. Ecological separation of systematically related stream insects. Canadian J. Zool. (In press.)

Holdsworth, R. P. 1941. The life history and growth of *Pteronarcys proteus*. Newman. Ann. Entomol. Soc. Am. 34: 495–502.

Hynes, H. B. N. 1958. The effect of drought on the fauna of a small mountain stream in Wales. Verhandl. Intern. Ver. Theort. Angew. Limnol. 13: 826–833.

1960. The biology of polluted waters. Liverpool Univ. Press, Liverpool. 202 p.

1961. The invertebrate fauna of a Welsh mountain stream. Arch. Hydrobiol. 57: 344–388.

1962. The significance of macro-invertebrates in the study of mild river pollution, p. 235–240. *In* C. M. Tarzwell [ed.] Third seminar in biological problems in water pollution. U.S. Public Health Serv. Publ. 999-WP-25. 424 p.

JONASSON, P. M. 1955. The efficiency of sieving techniques for sampling freshwater bottom fauna. Oikos 6: 183–207.

MACAN, T. T. 1958. Methods of sampling the bottom fauna in stony streams. Mitt. Intern. Ver. Limnol. 8: 1–21.
1963. Freshwater ecology. Longmans, Green and Co. Ltd., London. 338 p.

MACKAY, R. J., AND J. KALFF. 1969. Seasonal variation in standing crop and species diversity of insect communities in a small Quebec Stream. Ecology. (In press.)

MAITLAND, P. S. 1966. Studies on Loch Lomond. II. The fauna of the River Endrick. Blackie and Son Ltd., Glasgow. 194 p.

OLIVER, D. R. 1959. Some Diamesini (Chironomidae) from the Nearctic and Palearctic. Entomol. Tidsskr. 80: 48–64.

PATRICK, R. 1962. Algae as indicators of pollution, p. 225–230. *In* C. M. Tarzwell [ed.] Third seminar in biological problems in water pollution. U.S. Public Health Serv. Publ. 999-WP-25. 424 p.

ROOS, T. 1959. Studies on upstream migration in adult stream-dwelling insects. I. Rept. Inst. Freshwater Res., Drottningholm 38: 167–193.

ROSS, H. H. 1944. The caddis flies or Trichoptera of Illinois. Ill. Nat. Hist. Surv. Bull. 23: 10326.
1959. Trichoptera, p. 1024–1049. *In* W. T. Edmondson [ed.] Freshwater biology. John Wiley & Sons, Inc., New York. 1248 p.

SCOTT, D. 1958. Ecological studies on the Trichoptera of the River Dean, Cheshire. Arch. Hydrobiol. 54: 340–392.

SPRULES, W. M. 1947. An ecological investigation of stream insects in Algonquin Park, Ontario. Univ. Toronto Studies, Biol. Ser. 56: 1–88.

WIGGINS, G. B. 1960. A preliminary systematic study of the North American larvae of the caddis fly family Phryganeidae (Trichoptera). Canadian J. Zool. 38: 1153–1170.

WU, C. F. 1923. Morphology, anatomy and ethology of *Nemoura*. Lloyd Library, Cincinnati, Bull. 23: (Entomol. Ser. 3) 1–81.

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UV-Absorbing and UV-Fluorescing Substances in the Belly Skin of Fry of Coho Salmon (*Oncorhynchus kisutch*)

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The belly skin of 4-month-old coho salmon fry was extracted with 0.5 M NH_4OH, and the extract was analyzed by paper chromatography for UV-absorbing and UV-fluorescing substances. Six fluorescing and three absorbing substances were isolated and characterized by R*f* values, UV-absorption spectra, and chemical reactions, and were identified with known compounds where possible. The three UV-absorbing substances were identified as guanine, hypoxanthine, and inosine monophosphate. Three of the UV-fluorescing substances were identified as ichthyopterin, pterin 6-carboxylic acid, and 7,8-dihydroxanthopterin. A fourth fluorescing substance appeared to be an artefact produced by the action of NH_4OH on ichthyopterin. The remaining two fluorescing substances appeared to be unknown glycosides, presumably isomers, both containing glucose, xylose, and mannose.

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INTRODUCTION

A PRELIMINARY STUDY in this laboratory had indicated the presence of guanine, hypoxanthine, guanylic acid, and a number of unidentified UV-fluorescing substances in the belly skin of coho salmon fry (Markert and Vanstone, 1966). The fluorescent substances were believed to be pteridines, in view of several reports of their occurrence in fish skin and scales and of their involvement in pigmentation changes accompanying fish metamorphosis (Kauffmann, 1959a; Ziegler, 1963; Hama, 1963; Hama and Fukuda, 1964). The earlier work on coho salmon fry was extended by us in order to characterize and identify the fluorescent substances. Using milder extraction conditions and repeated paper-chromatographic analysis in a number of solvent systems, we have isolated six fluorescent and three UV-absorbing substances, the characterization and identification of which are described below.

MATERIALS AND METHODS

FISH SKIN

Four-month-old fry of coho salmon (*Oncorhynchus kisutch*) were collected from Big Qualicum River, Vancouver Island, B.C. All fish were anaesthetized, frozen and stored at –20 C. Rectangular sections of belly skin were removed from the partially thawed fish as previously described by Markert and Vanstone (1966).

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SKIN EXTRACTS

The skin sections were extracted in 0.5 M NH_4OH for 24 hr at room temperature (24–28 C) with frequent shaking, and the volume of the suspension was adjusted to contain approximately 125 mm² skin per ml. The extracts were filtered (Whatman No. 1), stored at 2 C, and analyzed as soon as possible.

PAPER CHROMATOGRAPHY

The extracts were applied as 2-inch streaks on Whatman No. 3MM chromatographic paper. Preliminary separation of the components was obtained by descending chromatography at room temperature, protected from direct light, for 24 hr in solvent system (I) organic upper layer of 4:1:5 (v/v) butan-1-ol:acetic acid:water. The developed chromatograms were air-dried at room temperature in the dark and scanned under UV-radiation at 366 and 253.7 mμ for UV-absorbing and -fluorescing spots. The substances from these areas were eluted with 0.5 M NH_4OH overnight at room temperature and the eluates were freeze-dried. The products were further purified by similar rechromatography in either solvent system (II) 170:41:39 (v/v) propan-2-ol: concentrated HCl:water, or (III) 2:1 (v/v) propan-1-ol:0.5 M NH_4OH.

Ascending paper chromatography on Whatman No. 3MM in the following solvent systems was also used for the characterization of the products and derivatives obtained from their reactions: (IV) 4% (w/v) aqueous sodium citrate; (V) 3% (w/v) aqueous NH_4Cl; (VI) 4:1:1 (v/v) butan-1-ol:acetic acid:water; (VII) 50:15:10:25 (v/v) ethanol:butan-1-ol:15 M NH_4OH:water. Authentic compounds were generally included, as standards on the chromatograms or for co-chromatography with the products where required.

ABSORPTION SPECTRA

All spectra were recorded on a Beckman DK-1 recording spectrophotometer in the range 220–400 mμ.

PAPER ELECTROPHORESIS

Aqueous solutions of compounds were applied as concentrated spots on Whatman No. 3MM paper strips and electrophoresis was conducted for 3 hr in a buffer solution of *p*H 8.6 contained in a Gelman Deluxe chamber and maintained under a gradient of 23 v/cm. The buffer solution was composed of 87 parts of 0.05 M sodium veronal and 13 parts of 0.05 N HCl. The developed electropherograms were air-dried at room temperature and scanned under UV-radiation as before.

CHEMICAL REACTIONS

PERIODATE OXIDATIONS

A solution of the compound (1 mg) in 2 N acetic acid (0.5 ml) was mixed with an equal volume of a similar solution of $NaIO_4$ (1 mg) and left standing at room temperature in the dark for 2 hr with occasional shaking. A suitable aliquot of the reaction mixture was then submitted to paper-chromatographic analysis of the products. For the oxidations under alkaline conditions, 2 N acetic acid was replaced by 0.2 M Na_2CO_3–$NaHCO_3$ buffer of *p*H 10.

H_2O_2 OXIDATIONS

A drop of 30% H_2O_2 was incorporated into a solution of the compound (0.5 mg) in 2 N acetic acid (1 ml) or into an equivalent amount of the above periodate oxidized reaction mixture. After standing at room temperature in the dark for 2 hr with occasional shaking, aliquots of each reaction mixture were submitted to paper-chromatographic analysis of the products.

PHOTOLYSIS EXPERIMENTS

A solution of the compound (1 mg) in 0.5 M NH_4OH (2 ml) was applied as concentrated spots on six Whatman No. 1 paper strips and air-dried. Pairs of the strips were next exposed to the following illumination conditions: (i) UV-lamp (366 and 253.7 mμ)[2] for 30 min; (ii) a 60-w incandescent lamp (illumination intensity, 140 ft-c) for 6 hr; (iii) usual laboratory lighting (illumination intensity, 15 ft-c) for 24 hr. One strip of each pair was then directly submitted to chromatographic analysis in one solvent system and its partner to similar analysis in a second solvent system.

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[2] "Chromato-Vue" of Ultra-violet Products, Inc., San Gabriel, California, with a distance of about 8 inches between lamp and paper.

MINERAL ACID HYDROLYSIS FOR CHARACTERIZATION OF GLYCOSIDES

The compound (0.5–1 mg) was heated with 1 N HCl (1 ml) in a sealed glass tube on a steam bath for 5 hr. The HCl was removed from the reaction mixture by repeated evaporation in vacuo with water. The dry residue was taken up in methanol and submitted to chromatographic analysis, alongside of sugar standards, on Whatman No. 1 paper in the following solvent systems: (VIII) 10:4:3 (v/v) ethyl acetate:pyridine: water; (IX) 18:3:1:4 (v/v) ethyl acetate:acetic acid: formic acid:water; and (X) upper layer of 4:1:5 (v/v) butan-1-ol:ethanol:water. The chromatograms were sprayed with 0.5% $AgNO_3$ solution in acetone, followed by 2% NaOH solution in ethanol, for the detection of sugars. A suitable portion of the acid hydrolysis product in methanol solution was also submitted to paper-chromatographic analysis for detection of UV-absorbing and -fluorescing substances.

AUTHENTIC COMPOUNDS

Reagent grade compounds or authentic specimens were obtained from the following sources and were used without further purification: Xanthopterin monohydrate and guanine from Mann Research Laboratories, New York; hypoxanthine and disodium inosine 5′-phosphate dihydrate from Pabst Laboratories, Milwaukee, Wisconsin; leucopterin from Calbiochem, Los Angeles, California; pterin-6-carboxylic acid, pterin-7-carboxylic acid, isoxanthopterin, and erythropterin monohydrate from Aldrich Chemical Co., Milwaukee, Wisconsin; neopterin from Dr M. Goto, Tokyo; isosepiapterin from Dr H. S. Forrest, Austin, Texas; and biopterin from Dr R. Tschesche, Bonn, Germany.

RESULTS

Chromatography of the skin extracts in solvent system I revealed six well-separated UV-detectable spots, four that were fluorescent and two that were UV-absorbing (Fig. 1I). On rechromatography in solvent system II, the compounds eluted from spots R1, R2, and R4 on the first chromatogram were further resolved into discrete pairs (R1A, R1B, R2A, R2B, R4A, and R4B, in Fig. 1II), R3 showed evidence of decomposition, R5 revealed two partially separated components, and R6 appeared to be homogeneous. When solvent system III was used after system 1, R3 showed both stability and homogeneity, R5 was clearly resolved into two well-defined components (R5A and R5B in Fig. 1III, and R1, R2, and R4 were again resolved into pairs of components but less efficiently than in solvent system II, and R6 again showed homogeneity. The 10 components (R1A, R1B, R2A, R2B, R3, R4A, R4B, R5A, R5B, R6) so isolated could not be further resolved on repeated chromatography in a number of solvent systems and were assumed to be homogeneous individuals. However, traces of R2B and R4A were frequently found accompanying the purified R2A and R4B respectively, and these were ultimately traced to chemical action of the eluting solvent on the latter compounds (see later sections). The chromatographic properties of the 10 compounds and their UV-absorption maxima are presented in Table 1, and their electrophoretic mobilities in Table 2.

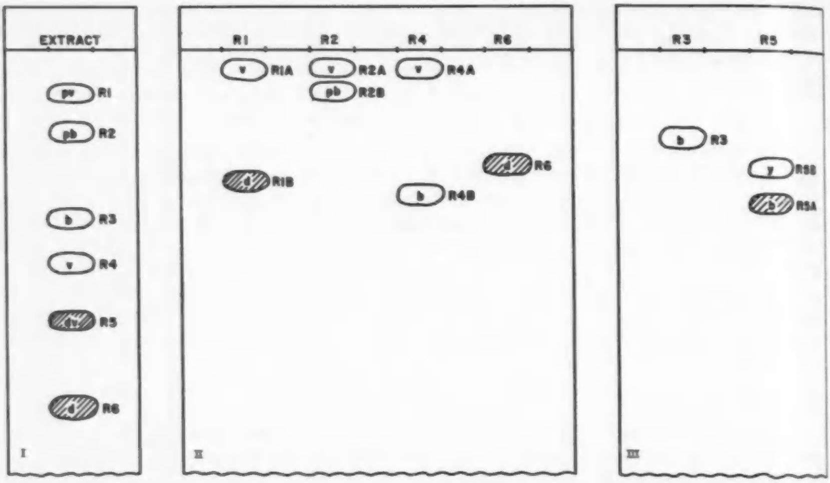


FIG. 1. Typical paper chromatograms of salmon skin UV-fluorescing and UV-absorbing substances: (I) aq NH_4OH extracts of skin developed with solvent system I (see text); (II) substances isolated from chromatogram (I) and re-chromatographed with solvent system II; (III) substances isolated from chromatogram (I) and re-chromatographed with solvent system III. Key to colors of UV-fluorescence (unhatched enclosures) and UV-absorption (hatched enclosures): *b*, blue; *v*, violet; *y*, yellow; *p*, pale; *d*, dark.

For further characterization and identification of these compounds, preparative-scale extractions from skin and chromatographic purification were undertaken as before, using the sequence of solvent systems I → II for the isolation of compounds R1A, R1B, R2A, R2B, R4A, R4B, and R6, and the sequence of solvent systems I → III for the compounds R3, R5A, and R5B. The compounds R1A, R1B, R2A, R2B, R4A, R4B, R5A, and R6 were obtained in crystalline form in quantities of several milligrams; R3 and R5B were isolated in amounts too low to permit crystallization and could only be characterized with eluate concentrates from chromatograms.

R1A

This blue-fluorescent compound showed chromatographic mobilities and UV-absorption spectra remarkably similar to those of R4A (see Table 1). The two compounds co-chromatographed without any detectable resolution and were, therefore, assumed to be identical. R1A may not be a native constituent of fish skin, since studies on the characterization of R4A (see later section) have shown that it is produced by the action of the extracting solvent (0.5 M NH_4OH) on R4B.

R1B

This compound appeared as a nonfluorescent, dark absorbing spot on chromatograms under the shorter wave-length UV-radiation. Its absorption

TABLE 1. Chromatographic properties and absorption spectra of UV-fluorescing and UV-absorbing substances isolated from coho salmon (fry) skin.

Isolated substances	Paper chromatographic properties: Color of fluorescence (*f*) or absorption (*q*) observed at 366 mμ	253.7 mμ	R_f values in solvent systems[a] I (↓)	II (↓)	III (↓)	IV (↑)	V (↑)	Absorption maxima[b] (mμ) in 0.1 N NaOH	0.1 N HCl	Known compounds with identical properties
R1A	Blue (*f*)	Nil	0.07	0.04	0.06	0.17	0.74	~274, 221	~274, 224	–
R1B	Nil	Violet (*q*)	0.07	0.32	0.12	–	–	254	250	Inosine 5′-phosphate
R2A	Violet (*f*)	Violet (*f*)	0.11	0.04	0.19	0.28	0.27	340, 276, 224	274, 219	–
R2B	Blue (*f*)	Blue (*f*)	0.11	0.08	0.25	0.28	0.28	340, 278, 224	272, 222	–
R3	” ”	Blue (*f*)	0.19	–	0.09	0.46	0.60	360, 262	330, 310, 260, 236	Pterin-6-carboxylic acid
R4A	” ”	Nil	0.07	0.05	0.06	0.17	0.74	~274, 221	~274, 224	–
R4B	” ”	Nil	0.24	0.34	0.20(↑)	0.54	0.56	340, 280, 258, 221	343, 286, 212	Ichthyopterin
R5A	Nil	Violet (*q*)	0.26	0.22	0.33	–	–	246, 273	249, 273	Guanine
R5B	Yellow (*f*)	Nil	0.26	0.23	0.28	0.40	0.70	278, 225	315, 278	7,8-Dihydroxanthopterin
R6	Nil	Violet (*q*)	0.34	0.29	0.64	–	0.56	261	249	Hypoxanthine

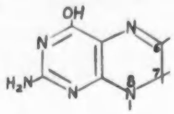
[a] See text for details of the solvent systems I–V; (↓) and (↑) denote descending and ascending chromatography, respectively, on Whatman No. 3MM paper.

[b] The symbol ~ denotes a shoulder (or inflexion) at the wavelength stated.

TABLE 2. Electrophoretic behaviour of coho salmon skin substances compared with known pteridines.

Compound	Pterin structure[a] with following substituents at				Electrophoresis in *p*H 8.6 veronal buffer, 23 v/cm, 3 hr Distance (*mm*) of movement towards	
	C_6	C_7	C_7—N_8 bond	N_8	Anode (+)	Cathode (−)
R1A (R4A)					7.8	–
R2A					23.5	–
R2B					23.5	–
Pterin-6-carboxylic acid (R3)	—COOH	—H	Double	Nil	67.0	–
Ichthyopterin (R4B)	$-\mathrm{CHOH \cdot CHOH \cdot CH_3}$	—OH	"	"	32.4	–
Isoxanthopterin	—H	—OH	"	"	36.0	–
Leucopterin	—OH	—OH	"	"	27.0	–
Erythropterin	—OH	$-\mathrm{CH{=}C}\genfrac{}{}{0pt}{}{\mathrm{OH}}{\mathrm{COOH}}$	"	"	47.0	–
Isosepiapterin	$-\mathrm{CO \cdot CH_2 \cdot CH_3}$	$\genfrac{}{}{0pt}{}{\mathrm{H}}{\mathrm{H}}$	Single	—H	–	8.0
H_2O_2 oxidation product of R4A					–	14.5

[a] Pterin structure:



spectra and R_f values (Table 1) closely corresponded to those obtained from an authentic specimen of inosine 5′-phosphate. The identity of R1B with inosine 5′-phosphate was confirmed by co-chromatography.

R2A AND R2B

These two colorless compounds appeared as bright fluorescent spots on chromatograms under both wavelengths of UV-radiation; R2A fluoresced violet and R2B pale blue. They exhibited virtually identical R_f values in solvent systems I, IV, and V, but were resolvable in systems II and III. Each compound showed considerably different spectra in acidic and alkaline solution, but the spectra of both compounds were virtually identical at each *p*H (see Table 1). Furthermore, they displayed identical electrophoretic migration towards the anode (see Table 2). These observations suggested that the two compounds were probably isomeric and that the extraction or elution conditions used may have caused their interconversion. Indeed, R2A was found to change to R2B on dissolution in 0.5 M NH_4OH and even in water; the change was rapid enough to be detectable within 1 hr of dissolution and complete conversion was obtained on standing overnight at room temperature. The interconversion appeared to proceed only in the direction R2A → R2B, since R2A was never detected in solutions of R2B under any of the conditions examined. No conversion of R2A was detected in methanol solution and the absorption spectra of both compounds in methanol were again identical (λ_{max} ~ 232, 223, 218).

The *p*H- or water-dependent nature of the conversion observed was reminiscent of the mutarotation and anomerization of sugars. Mineral acid hydrolysis tests for glycosidic sugar indicated that both R2A and R2B may contain sugars with R_f values in close agreement with those of D-glucose, D-xylose, and D-mannose in three solvent systems (VIII, IX, X), which are known to differentiate the common, naturally occurring monosaccharides diagnostically (J. N. C. Whyte, unpublished information, 1968). Visual inspection of the color intensities obtained on the chromatograms suggested that the sugars may be present in the following order of concentration: glucose > xylose > mannose.

Chromatographic analysis of the aglycones (from mineral acid hydrolyzates of R2A and R2B) in solvent system I revealed three fluorescent spots with the following characteristics: (i) pale yellow, R_f 0.51 (presumably the aglycone); (ii) dark violet, R_f 0.09 (presumably unhydrolyzed R2A or R2B); (iii) blue, R_f 0.00 (presumably an artefact). The observed chromatographic properties of the aglycone suggested a similarity to only xanthopterin among the known pteridines (Hirata et al., 1950). However, similar mineral acid treatment and chromatographic analysis of authentic xanthopterin gave three different products (yellow spot of R_f 0.35, violet spot of R_f 0.24, and pale-blue spot of R_f 0.06). The aglycones from R2A and R2B also did not appear to be flavonoids, since they failed to react on paper with the following flavonoid-specific spray reagents: Benedict's solution (Vogel, 1957), diazotized *p*-nitroaniline (Swain, 1953), and 1:1 (v/v) mixture of 1% $FeCl_3$ and 1% $K_3Fe(CN)_6$ (Barton et al., 1952).

Melting point determinations (with Kofler block) on crystals of R2A and R2B showed onset of charring at 165–170 C, disappearance of the charring at 230–235 C, and no melting of the compounds up to 350 C. Attempted determinations of infrared spectra in nujol mull gave poor resolution. Further characterization of the compounds was not possible, owing to lack of sufficient material.

R3

This blue-fluorescent compound occurred in trace amounts on chromatograms of skin extracts. Its chromatographic properties and absorption spectra (Table 1) corresponded closely with those previously reported for 2-amino-4-hydroxypteridine-6-carboxylic acid (pterin-6-carboxylic acid) (Levy, 1964; Kobayashi and Forrest, 1967; Watt, 1967; Goto et al., 1965). Chromatography of authentic pterin-6-carboxylic acid in solvent systems I, III, IV, and V, and co-chromatography with R3 confirmed the identity of the two compounds.

R4A AND R4B

The compound R4B appeared to be the principal fluorescent component of the skin extracts; it fluoresced blue to violet on paper chromatograms and was obtained as a white crystalline powder (mp > 350 C). Its chromatographic properties and absorption spectra (Table 1) corresponded closely with those previously reported for ichthyopterin (Tschesche and Korte, 1951; Kauffmann, 1959b; Matsumoto et al., 1960; Hüttel and Schreck, 1960; Kushibiki et al., 1954; Mori et al., 1960). However, in the absence of authentic ichthyopterin, further characterization of R4B was required to confirm its identity. The characterization was based on the peculiar chemical properties of ichthyopterin known to possess the structure of 7-hydroxybiopterin or 6-(α,β-dihydroxypropyl)-isoxanthopterin (Kauffmann, 1959b; Tschesche and Glaser, 1958) (see Fig. 2). Periodate oxidation of R4B in 2 N acetic acid,

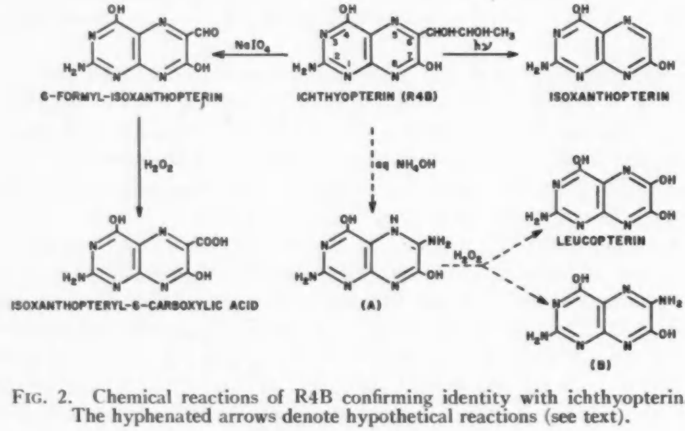


FIG. 2. Chemical reactions of R4B confirming identity with ichthyopterin. The hyphenated arrows denote hypothetical reactions (see text).

followed by chromatographic analyses in solvent systems V and VI, gave a blue-fluorescent product with R_f values of 0.25 and 0.13, respectively, in good agreement with those previously reported for 6-formyl-isoxanthopterin formed by similar oxidation of ichthyopterin (Kauffmann, 1959b; Tschesche and Glaser, 1958). When the periodate oxidation was followed in situ by H_2O_2 oxidation, chromatographic analyses in solvent systems V and IV gave a fluorescent product with R_f values of 0.38 and 0.20, respectively, in good agreement with those previously reported for the expected isoxanthopteryl-6-carboxylic acid (Matsumoto et al., 1960; Kushibiki et al., 1954). Furthermore, the photolysis tests also showed R4B to be photolabile in a manner similar to that previously reported for ichthyopterin. Chromatography of the UV-irradiated material in solvent system VII showed complete conversion of R4B (R_f, 0.30) to a fluorescent product corresponding in R_f value (0.25) to authentic isoxanthopterin. A similar UV-induced photolysis of ichthyopterin to isoxanthopterin was noted by Tschesche and Glaser (1958). Under incandescent-light irradiation, R4B gave evidence of partial photolysis to two unidentified blue-fluorescent products of R_f 0.15 and 0, with no detectable formation of isoxanthopterin. Kauffmann (1959b) obtained similar unidentified photolysis products from exposure of ichthyopterin to natural light from a northern window. R4B was recovered unchanged, with no evidence of photolysis, from exposure to normal laboratory lighting conditions, indicating that these conditions were satisfactory for extraction and isolation of the compound without decomposition. It was concluded from the overall similarity observed in all their properties tested that R4B must be identical with ichthyopterin.

The violet-fluorescent compound R4A (white crystals, mp > 350 C) was shown to be identical with R1A (see earlier section)and differed widely in chromatographic mobility and absorption spectra from R4B (see Table 1). However, it was always found to accompany R4B, even after repeated chromatography in solvent systems (such as I and II) known to give excellent separation of the two compounds (see Fig. 1). This indicated that R4A was an artefact formed from R4B under the experimental conditions used. The photolysis studies on R4B had not shown R4A as a product. The role of the extraction (and eluting) solvent commonly used was next investigated, and it was indeed found that R4B gave rise to R4A on extraction with 0.5 M NH_4OH, but not with water or methanol. Treatment of R4B with 1 N NaOH gave no evidence of formation of R4A, indicating that hydroxyl ions were not involved in the artefact formation. From these observations, it was concluded that R4A was a product of the action of NH_3 (or NH_4^+) on ichthyopterin (R4B).

Unlike ichthyopterin, R4A appeared unchanged on chromatograms after treatment with periodate in 2 N acetic acid or in *p*H 10 buffer. This indicated that R4A lacked the vicinal diol group in a side chain. The possibility of an α-amino alcohol group having arisen from the action of ammonia on the ichthyopterin side chain appeared to be unlikely from the observed inertness of R4A to periodate in alkaline solution, since studies on sphingosine have shown the grouping — $CHOH{\cdot}CHNH^2{\cdot}CH^2OH$ to be cleaved by periodate in ealkalin medium, although not in acid (Murray, MS, 1947). R4A also emerged unchanged from the photolysis tests causing decomposition of ichthyopterin. Since the photolability of ichthyopterin and the closely related biopterin are attributed to a reactive α,β-dihydroxypropyl side chain at C_6 in both compounds (Mori et al., 1960), it appeared that this side chain had undergone an unusual chemical transformation or, more likely, was split from the action of ammonia on ichthyopterin. R4A was oxidized by H_2O_2 in 2 N acetic acid to a single violet-fluorescent product with R_f values of 0.78, 0.80, and 0.50, in solvent systems V, IV, and VI respectively. Since the latter two values corresponded closely to those previously reported for α-(isoxanthopteryl-6-)propionic acid (Matsuura et al., 1953), it was thought that R4A might have been the corresponding aldehyde. However, R4A gave a negative reaction to Schiff's fuchsin reagent for aldehydes (Vogel, 1957). The cathodic migration of the oxidation product in contrast to the anodic migration of R4A during electrophoresis (Table 2) also argued against the conversion —CHO → —COOH. The oxidation product showed absorption spectra (λ_{max} ~ 252, 224 mμ in 0.1 N NaOH; ~ 256, 223 mμ in 0.1 N HCl) akin to those of R4A (Table 1). Further characterization of R4A was not possible, owing to shortage of material.

R5A

This major component appeared as a nonfluorescent, violet-blue absorbing spot on paper chromatograms. Its chromatographic mobility and absorption spectra in acid and alkali (Table 1) corresponded closely with those of guanine (Chargaff and Davidson, 1955), expected to occur in the skin extracts from our earlier study (Markert and Vanstone, 1966). Co-chromatography with authentic guanine confirmed the identity of R5A with the purine.

R5B

This yellow-fluorescent minor component displayed chromatographic properties resembling those of xanthopterin, but differed markedly in absorption spectra (Table 1), the nature of which suggested a dihydroxanthopterin. The paper chromatographic similarity of dihydroxanthopterin to xanthopterin has been previously noted (Renfrew and Piatt, 1950), and absorption spectra previously reported for 7,8-dihydroxanthopterin (Stuart et al., 1964) corresponded closely with those obtained from R5B. In order to confirm the suggested identity of R5B with dihydroxanthopterin, both R5B and authentic xanthopterin were submitted to treatment with H_2O_2 in 0.1 N NaOH solution for 24 hr at room temperature. Chromatographic analyses of the products, after neutralization (with HCl) and concentration, revealed only one fluorescent compound in each case with identical R_f values (0.34 in solvent system I, 0.35 in solvent system V) and absorption spectra (λ_{max} 255, 392 mμ in 0.1 N NaOH) characteristic of xanthopterin (Anderson and Nelson, 1949). This indicated that H_2O_2 had oxidized R5B to xanthopterin without effect on the latter compound. A similar oxidation of 7,8-dihydroxanthopterin to xanthopterin has been previously reported by Forrest et al. (1966). It was tentatively concluded from the evidence obtained that R5B may be identical with 7,8-dihydroxanthopterin.

R6

This component appeared as a dark-violet nonfluorescent spot on chromatograms and showed close correspondence in R_f values and absorption maxima (Table 1) to those previously reported for hypoxanthine (Chargaff and Davidson, 1955; Harmsen, 1966; Ikan and Ishay, 1967). Co-chromatography with authentic hypoxanthine confirmed the identity of R6 with the purine, and also verified its detection in the skin extracts previously studied in this laboratory.

DISCUSSION

Of the nine compounds isolated from the skin extracts, two (R1A($\equiv$R4A) and R2B) appear to be artefacts from the action of extracting solvent (aq NH_4OH) on the corresponding native components (R4B and R2A respectively). R1B, R5A, and R6 have been definitely identified with the purines inosine 5′-phosphate, guanine, and hypoxanthine respectively. R3 and R4B have been almost certainly identified with the pteridines pterin-6-carboxylic acid and ichthyopterin respectively, and R5B tentatively with 7,8-dihydroxanthopterin. The identity of R2A and of the artefacts R2B and R1A remain unestablished, although the characterization studies have shed considerable light on their chemical structure.

R2A and R2B were characterized as UV-fluorescing, colorless, nonflavonoid glycosides, containing glucose, xylose, and mannose. The nature of their absorption spectra and changes in maxima obtained with *p*H are strongly suggestive of pteridine structures (Mason, 1954). Further characterization of these compounds would be extremely interesting, since the few naturally occurring pteridine glycosides hitherto reported (Hatfield et al., 1961; Pfleiderer, 1964) were found in algae and each showed only one component sugar (glucose, galactose, quinovose, xylouronic acid, xylose, or ribose). The overall similarity displayed by R2A and R2B in all their properties examined indicates a close similarity in their structures, and the mild nature of the chemical conditions causing their interconversion suggests that they may be isomeric (probably anomeric) compounds. The facile and irreversible nature of the observed R2A → R2B conversion further suggests that the labile R2A is the naturally occurring compound and that the stable R2B may be an artefact of extraction and isolation procedures.

The characteristic chemical reactions confirming the identity of R4B with ichthyopterin are depicted in Fig. 2. Since the artefact R4A proved inert to both periodate and light, it became obvious that the action of ammonia had radically modified or eliminated the C_6 side chain of ichthyopterin. Aqueous ammonia and nucleophilic reagents have been known to add across the $N_5 - C_6$ bond of folic acid, biopterin, and other pteridines, leading in the case of NH_3

to loss of the C_6 side chain and formation of 6-amino derivatives (Van Baalen and Forrest, 1959; Vonderschmitt et al., 1967). An analogous action of ammonia on ichthyopterin may be expected to produce the structure A (Fig. 2), which may be oxidized by H_2O_2 to structure B and leucopterin. However, some of our observations appear to argue against the probability of structure A for R4A. The oxidation of R4A produced no detectable leucopterin. Also, structure A would be more basic than either ichthyopterin or structure B, whereas the electrophoretic mobilities observed (Table 2) indicate the following order of basicity: ichthyopterin $<$ R4A $<$ H_2O_2 oxidation product of R4A. It is interesting to note from the electrophoretic picture of Table 2 that the anode-oriented movement of ichthyopterin (comparable to that of isoxanthopterin) has been reversed by the sequence of reactions involving ammonia and H_2O_2 to a cathode-oriented movement of the R4A-oxidation product (comparable to that of isosepiapterin).

Of the three purines tentatively identified in our earlier study, the occurrence of guanine and hypoxanthine in the belly skin of coho salmon fry is confirmed by the present investigation. In addition, inosine monophosphate was isolated. The earlier-reported guanylic acid could not be detected. This discrepancy may be due to age-dependent physiological changes known to occur in immature fish (Barrington, 1961). The occurrence of guanine and hypoxanthine has been previously recorded for other species of fish (Johnston and Eales, 1967; Ziegler-Günder, 1956), but not the occurrence of inosine monophosphate.[3] It is quite possible that this nucleotide may have arisen from thin layers of muscle attached to the belly skin. Our finding of ichthyopterin as the principal skin pteridine in coho salmon is in general accord with previous observations on other fish species (Kauffmann, 1959a; Matsumoto et al., 1960). On the other hand, pterin-6-carboxylic acid has been only occasionally observed as a minor component in some species (Hama, 1963; Matsumoto et al., 1960); dihydroxanthopterin has not been isolated from any fish, although it has been assumed to play a biochemical role in melanophore development and differentiation (Hama and Fukuda, 1964; Ziegler, 1963). It is interesting to note that none of the pteridines (isoxanthopterin, isoxanthopterin-6-carboxylic acid, etc.) normally associated with ichthyopterin metabolism were found by us, in contradistinction to most earlier work (Hama, 1963; Hama and Fukuda, 1964; Kauffmann, 1959a). The absence of biopterin and sepiapterins found in most other fish examined (Hama, 1963; Hama and Fukuda, 1964) may reflect a species characteristic or a particular phase in the physiology of growth of coho salmon. The possibility is not precluded that the acetylated pteridines Cyprino Purple C_1 and C_2, reported to occur in several fish species, may have been converted to ichthyopterin by our method of extraction (Ohta and Goto, 1968). In concluding, it may be pointed out that several of the above discussed differences in pteridine composition may be due to undefined areas of skin used by other workers, since our preliminary examination of the same fish used in the present investigation has shown a more complex mixture of UV-fluorescing compounds in the dorsal skin than that reported here (unpublished observations).

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[3] NOTE ADDED IN PROOF: More recently, Hayashi and Saito (1968) have reported the occurrence of inosine monophosphate in the skin of carp, rainbow trout, and salmon.

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REFERENCES

Anderson, A. G., Jr., and J. A. Nelson. 1949. Purification of xanthopterin. J. Am. Chem. Soc. 71: 3837–3838.

Barrington, E. J. W. 1961. Metamorphic processes in fishes and lampreys. Am. Zool. 1: 97–106.

Barton, G. M., R. S. Evans, and J. A. F. Gardner. 1952. Paper chromatography of phenolic substances. Nature 170: 249–250.

Chargaff, E., and J. N. Davidson. 1955. The nucleic acids, vol. I. Academic Press, Inc., New York. p. 252, 493.

Forrest, H. S., M. Menaker, and J. Alexander. 1966. Studies on the pteridines in the milkweed bug, *Oncopeltus fasciatus* (Dallas). J. Insect Physiol. 12: 1411–1421.

Goto, M., H. S. Forrest, L. H. Dickerman, and T. Urushibara. 1965. Isolation of a new naturally-occurring pteridine from bacteria, and its relation to folic acid biosynthesis. Arch. Biochem. Biophys. 111: 8–14.

Hama, T. 1963. The relation between the chromatophores and pterin compounds. Ann. N.Y. Acad. Sci., 100: 977–986.

Hama, T., and S. Fukuda. 1964. The role of pteridines in pigmentation, p. 495–505. *In* W. Pfleiderer and E. C. Taylor [ed.] Pteridine chemistry. Pergamon Press Limited, Oxford.

Harmsen, R. 1966. Identification of fluorescing and UV-absorbing substances in *Pieris brassicae* L. J. Insect Physiol. 12: 23–30.

Hatfield, D. L., C. Van Baalen, and H. S. Forrest. 1961. Pteridines in blue-green algae. Plant Physiol. 36: 240–243.

Hayashi, S., and T. Saito. 1968. Biochemical studies on the skin of fish — I. Acid-soluble nucleotides in the dermis of some fishes. Bull. Japan. Soc. Sci. Fish. 34: 816–825.

Hirata, Y., K. Nakanishi, and H. Kikkawa. 1950. Xanthopterin obtained from the skins of the yellow mutant of *Bombyx mori* (silkworm). Science 111: 608–609.

Hüttel, R., and D. Schreck. 1960. Notiz über Ichthyopterin. Chem. Ber. 93: 2439–2441.

Ikan, R., and J. Ishay. 1967. Pteridines and purines of the queens of the oriental hornet, *Vespa orientalis* F. J. Insect Physiol. 13: 159–162.

Johnston, C. E., and J. G. Eales. 1967. Purines in the integument of the Atlantic salmon (*Salmo salar*) during parr–smolt transformation. J. Fish. Res. Bd. Canada 24: 955–964.

Kauffmann, T. 1959a. Untersuchungen über die fluoreszierenden Pigmente in der Haut von Zierfischen. Z. Naturforsch. 14B: 358–363.

1959b. Notiz über die Konstitution des Ichthyopterins. Liebigs Ann. Chem. 625: 133–139.

Kobayashi, K., and H. S. Forrest. 1967. Identification of pteridines produced by three species of photosynthetic bacteria. Biochim. Biophys. Acta 141: 642–644.

Kushibiki, K., T. Hama, and T. Goto. 1954. Quelques ptérines isolées de la peau ou des écailles de la carpe et leur transformation photochimique. Compt. Rend. Soc. Biol. 148: 759–762.

Levy, C. C. 1964. Pteridine metabolism in the skin of the tadpole *Rana catesbeiana*. J. Biol. Chem. 239: 560–566.

Markert, J. R., and W. E. Vanstone. 1966. Pigments in the belly skin of coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Bd. Canada 23: 1095–1098.

Mason, S. F. 1954. Some aspects of the ultraviolet absorption spectra of the pteridines, p. 74–92. *In* G. E. W. Wolstenholme and M. P. Cameron [ed.] Ciba Foundation symposium on chemistry and biology of pteridines. J. and A. Churchill Ltd., London.

Matsumoto, J., T. Kajishima, and T. Hama. 1960. Relation between the pigmentation and pterin derivatives of chromatophores during development in the normal black and transparent scaled types of goldfish (*Carassius auratus*). Genetics 45: 1177–1189.

Matsuura, S., S. Nawa, H. Kakizawa, and Y. Hirata. 1953. Studies on pteridines, IV; synthesis of 6- and 7-hydroxypteridines. J. Am. Chem. Soc. 75: 4446–4449.

Mori, Y., J. Matsumoto, and T. Hama. 1960. On the properties of Cyprino-pourpre A 2, a pterin isolated from the skin of Cyprinidae, and its relation to ichthyopterin or 7-hydroxybiopterin. Z. Vergleich. Physiol. 43: 531–543.

Murray, M. J. MS, 1947. Studies on sphingosine. M.A. Thesis. University of Toronto, Toronto, Ont.

Ohta, K., and M. Goto. 1968. Isolation and identification of acetylated pteridines from loach. J. Biochem. (Tokyo) 63: 127–129.

Pfleiderer, W. 1964. Recent developments in the chemistry of pteridines. Angew. Chem. Intern. Ed. Engl. 3: 114–132.

Renfrew, A. G., and P. C. Piatt. 1950. Paper chromatography of some synthetic pteridines. J. Am. Pharm. Assoc. 39: 657–659.

Stuart, A., D. W. West, and H. C. S. Wood. 1964. Pteridine derivatives, Part IX; 2,6-diamino-4-hydroxypteridine and related dihydropteridines. J. Chem. Soc. 1964: 4769–4774.

Swain, T. 1953. The identification of coumarins and related compounds by filter-paper chromatography. Biochem. J. 53: 200–208.

Tschesche, R., and A. Glaser. 1958. Über Pteridine, XVI, Synthesen des 7-Hydroxybiopterins und über seine Beziehungen zum Ichthyopterin. Chem. Ber. 91: 2081–2089.

Tschesche, R., and F. Korte. 1951. Über Pteridine, V, Mitteil.: die Konstitution des Ichthyopterins. Chem. Ber. 84: 801–809.

Van Baalen, C., and H. S. Forrest. 1959. 2,6-Diamino-4-hydroxypteridine, a new naturally-occurring pteridine. J. Am. Chem. Soc. 81: 1770–1771.

Vogel, A. I. 1957. Practical organic chemistry, 3rd ed. Longmans, Green and Co., Ltd. London. p. 454, 330.

Vonderschmitt, D. J., K. S. Vitols, F. M. Huennekens, and K. G. Scrimgeour. 1967. Addition of bisulfite to folate and dihydrofolate. Arch. Biochem. Biophys. 122: 488–493.

Watt, W. B. 1967. Pteridine biosynthesis in the butterfly *Colias eurytheme*. J. Biol. Chem. 242: 565–572.

Ziegler, I. 1963. Tetrahydropterin und Melanophoren-differenzierung bei Fischen. Z. Naturforsch. 18B: 551–556.

Ziegler-Günder, I. 1956. Untersuchungen über die Purin- und Pterinpigmente in der Haut und in den Augen der Weissfische. Z. Vergleich. Physiol. 39: 163–189.

Age Determination of Sockeye (*Oncorhynchus nerka*) and Chum (*O. keta*) Salmon from Examination of Pectoral Fin Rays

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Ages of adult sockeye and chum salmon were estimated from examination of the second anterior pectoral fin ray, and were compared with estimates from their scales or otoliths. For sockeye, only the ocean age could be estimated. Freshwater annual rings could not be distinguished on their fin rays even though they were present on their scales or otoliths. In general, the three methods provided the same estimates of ocean age. Agreement of estimates of ocean age by one to five readers ranged from 78.9 to 98.8%. For chum salmon, the scale and fin ray methods generally provided the same estimates of total age. Agreement of estimates of total age by one or two readers ranged from 73.8 to 99.3%.

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INTRODUCTION

The age of Pacific salmon is most often estimated by examination of scales. As the salmon approach maturity, however, the scales are absorbed until only the centre portion remains; hence total age cannot be estimated from the scale markings. The age of salmon may also be estimated by examination of otoliths (Clutter and Whitesel, 1956; Bilton and Jenkinson, 1968). Ages obtained by this method usually are similar to those estimated from scales, but the disadvantage is that the fish must be sacrificed. This restricts the application, for salmon, to sampling of commercial catches or to fish that have spawned and died. Age may be estimated also from length distribution data, although precision is lessened by a rather wide overlap of length frequency among age groups.

A fourth method of estimating age of fishes is by examination of their fin rays, which, like their scales, may be obtained without killing the donor. Pectoral fin rays of among others, lake sturgeon (*Acipenser fulvescens*), lake trout (*Salvelinus namaycush*), mountain whitefish (*Prosopium williamsoni*), cutthroat trout (*Salmo clarki*), rainbow trout (*Salmo gairdneri*), and white sucker (*Catostomus commersoni*) have been used for this purpose (Cuerrier, 1951; Scidmore and Glass, 1953; Chugunova, 1959).

To our knowledge this method has not been applied to aging any of the five species of Pacific salmon. The purpose of this paper is to describe a method of estimating sockeye and chum salmon ages from examination of the second

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anterior pectoral ray and to compare these estimates to those from their scales or otoliths.

MATERIALS AND METHODS

In 1966, 1967, and 1968, left pectoral fins and either scales or otoliths were removed from sockeye and chum salmon as detailed in Table 1. The majority of the fins were removed at the base including the articulation (Fig. 1*a*), and the remainder distal to the articulation.

In the laboratory, plastic impressions were made of the scales, and the otoliths were placed in glycerin. Otoliths were examined under a binocular microscope over a black background using reflected light. The scales were examined as projected images at 100 magnifications. The fins were dried. Although rays 2–4 near the anterior edge of the fin appeared to be most suitable for aging, to provide consistency only the second ray was used. The rays were sectioned using a high-speed circular saw built for the purpose. Although generally it has been the practice to section rays for aging (Cuerrier, 1951; Chugunova, 1959; Boyko, 1946), in the present study we found that features of the ray were just as clearly defined on the cut end of the base of the whole ray as in a thin section. All ages were determined from the cut end of the whole ray. The ray was cut at right angles to its axis at the widest point near the basal end (Fig. 1*b*), and the end of the ray was dipped into a clearing agent and placed upright in a special holder (Fig. 2). The cut end was examined under a binocular microscope against the black background of the holder, using a narrow beam of reflected light. The best clearing agent was a light lubricating oil, such as sewing machine oil. Xylol evaporated too quickly; water and glycerin did not provide sufficient contrast between light and dark areas.

Under the microscope, wide opaque zones alternating with narrow translucent zones were seen on the end of the fin ray (Fig. 3). By analogy with otoliths, the opaque zone or band was presumed to represent material deposited during the summer and the translucent band material deposited during the winter, thus being analogous to an annual ring of an otolith and an annulus of a scale (Fig. 4).

For sockeye, which usually spend one or two of their first years in fresh water before migrating to sea, total age could not be determined from the fin rays. Freshwater annual rings could not be distinguished either on sections or on whole rays, even though scales or otoliths indicated that most of the fish had spent one winter, and some two winters, in fresh water (Fig. 4). Techniques similar to those described by Chugunova (1959) for mounting and sectioning rays of small fish were used. A freshwater annual ring could not be detected even on fin rays of 1-year-old sockeye smolts (age as determined from their scales). Further evidence to support this assertion, that the first annual ring on sockeye fin rays is not of freshwater origin but of ocean origin, is obtained from comparisons of rays of pink salmon with those of a one-ocean-annulus sockeye. Since virtually all pinks spend one winter in the ocean (Bilton and Ricker, 1965), and none in fresh water after hatching, only one annual ocean ring would be expected (Fig. 5). The position of this ring on the ray was approximately the same for both species. As a consequence, it appears that only the number of winters spent in the ocean could be determined from the fin rays. By the same token, total age of chum could be determined from the fin rays because chum migrate to sea as fry shortly after emergence. Examination of their scales and otoliths indicates an absence of freshwater annual rings, and the presence of ocean annual rings only (Fig. 4).

Aging of each fish by fin rays and either scales or otoliths was done at different times. Some fish were first aged from their scales or otoliths, and others first from their fin rays. In no instance were the ages previously determined from the scales, otoliths, or fin rays referred to when the samples were subsequently aged by an alternative method. Sockeye fins from Babine Lake spawners were examined by five readers. Fins from sockeye and chums from other areas were examined by one or two readers.

RESULTS

Estimates of ages made by using fin rays and either scales or otoliths are compared in Table 1. For the sockeye it is apparent that the three methods

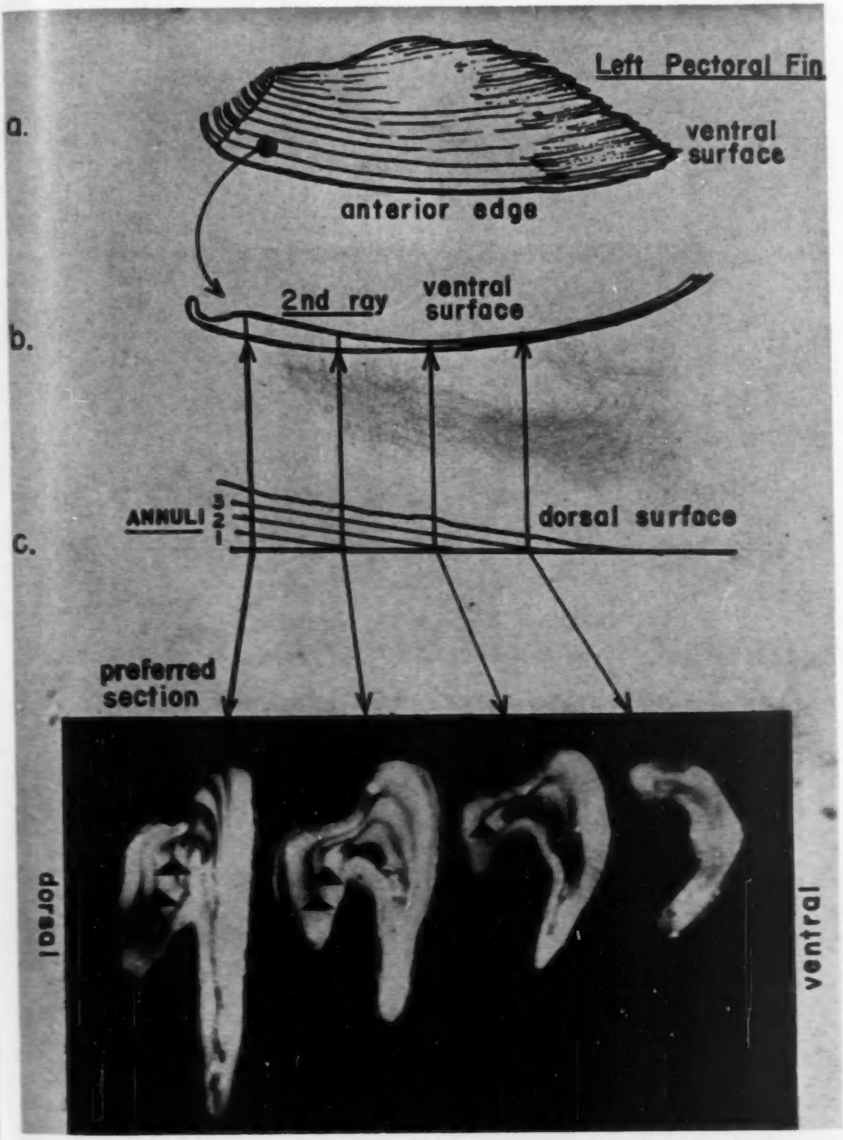
Left Pectoral Fin
a.
ventral surface
anterior edge
2nd ray
ventral surface
b.
ANNULI
3
2
1
dorsal surface
c.
preferred section
dorsal
ventral

FIG. 1. (*a*) Diagram of pectoral fin from sockeye salmon; arrow indicates the preferred second anterior ray. (*b*) Second anterior ray; shows position of preferred section. (*c*) Sections from various positions on second ray.

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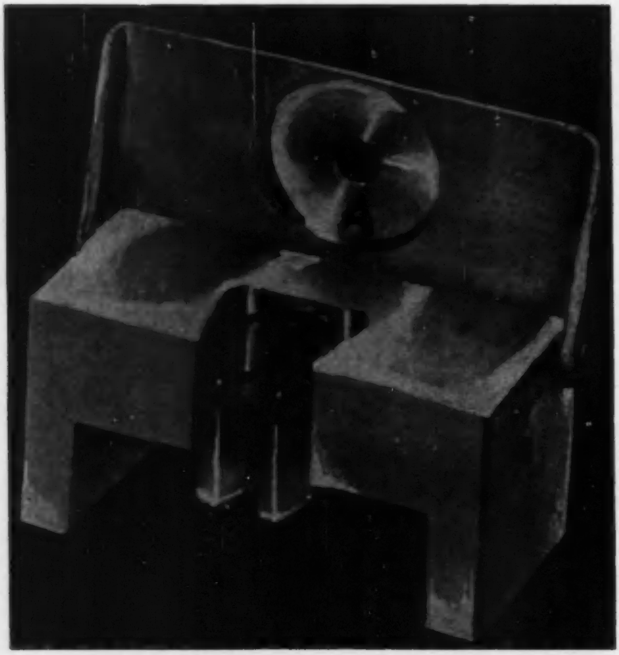

FIG. 2. Holder for examining fin rays: (A) recessed for circular cover glass; and (B) plastic clip for holding fin ray section in upright position.

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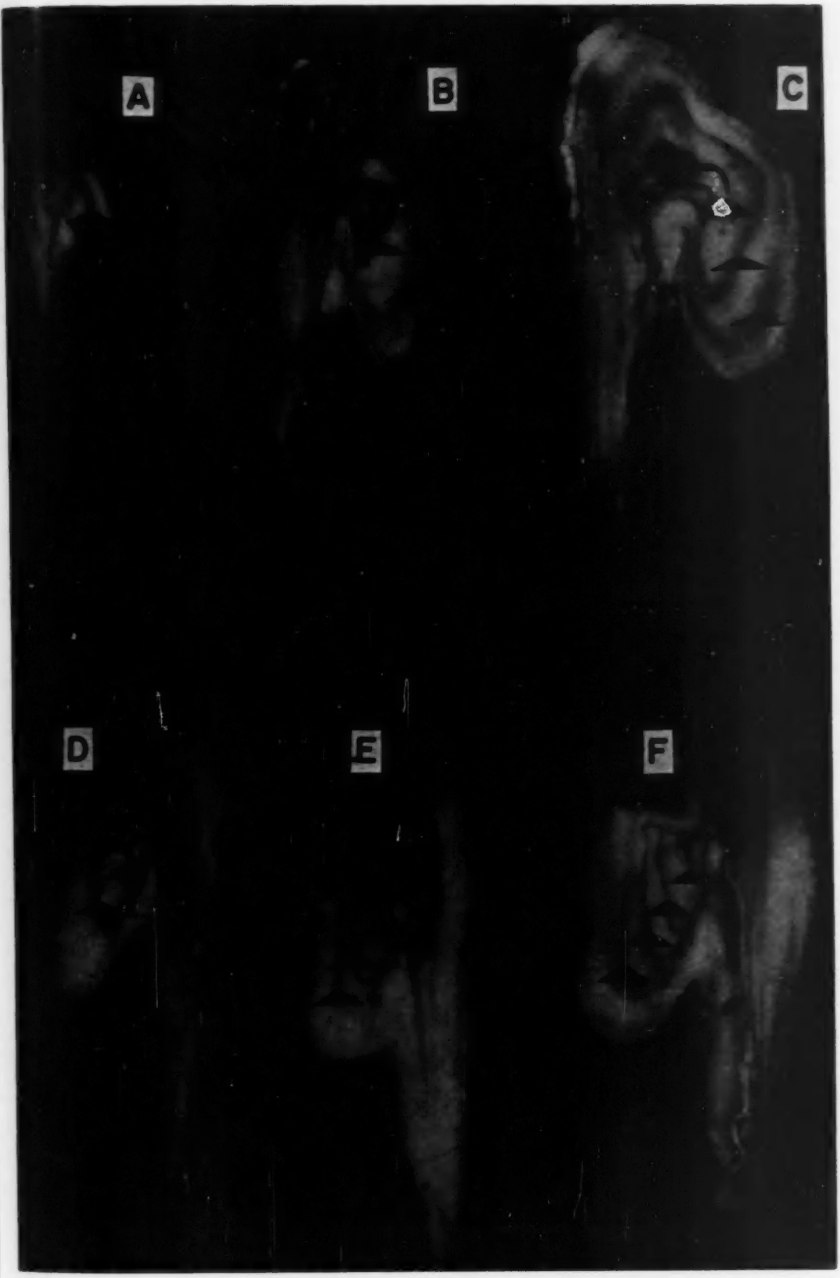

FIG. 3. Fin ray sections A, B, and C from age .1, .2, and .3 sockeye salmon; sections D, E, and F from 0.2, 0.3, and 0.4 chum salmon.

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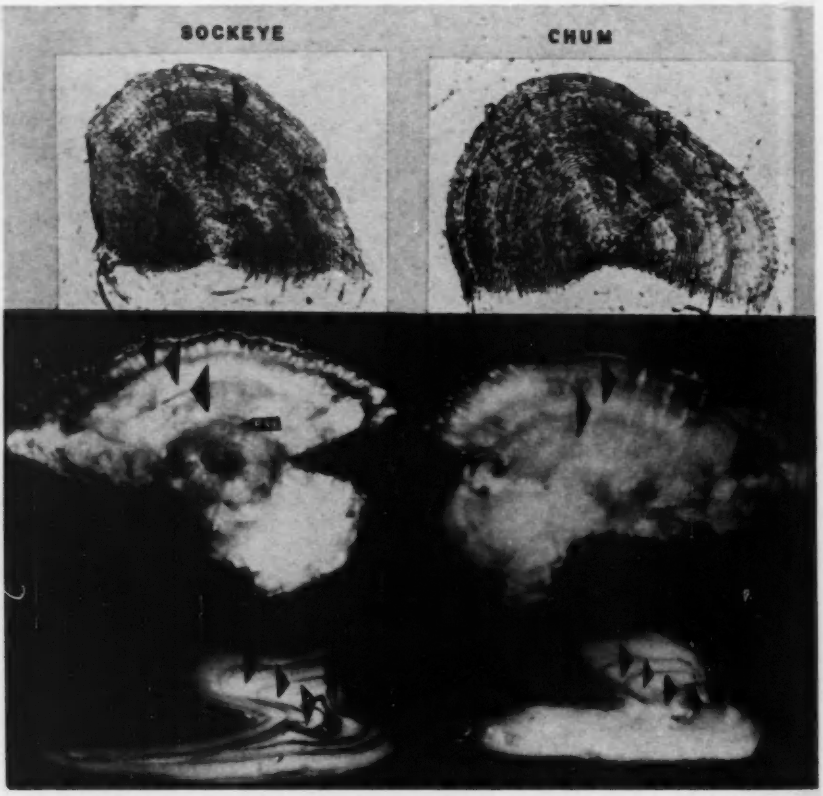

FIG. 4. Scale, otolith, and fin ray from a 1.3 sockeye and a 0.3 chum salmon: FW indicates freshwater annulus; arrows indicate ocean annuli.

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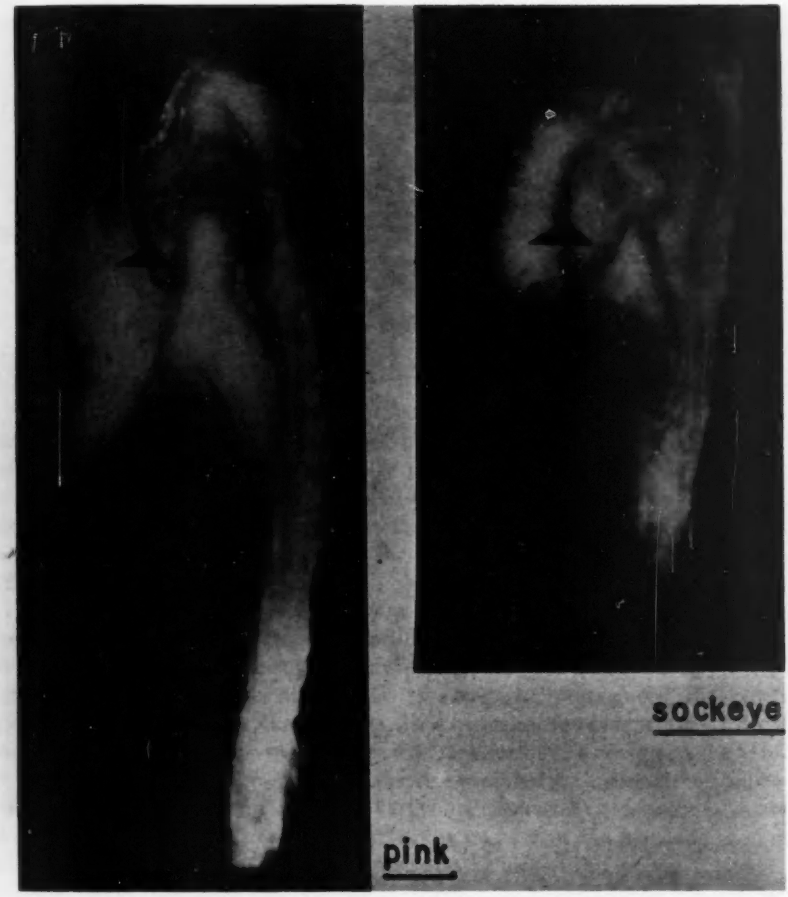
pink
sockeye

FIG. 5. Fin ray sections from a 0.1 pink salmon and a .1 sockeye salmon.

TABLE

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TABLE 1. Origin and numbers of sockeye and chum salmon employed in age determinations and the percentage agreement between fin rays and either scales or otoliths.

Species	Location	Year	Total no. sampled	No. all aged	Comparison between	Reader no.	Age agreed No.	Age agreed %
Sockeye	Babine Lake spawners	1967	102	82	Otolith and fin	1	76	92.7
						2	74	90.2
						3	81	98.8
						4	71	86.6
						5	78	95.1
	Skeena River Estuary	1968	50	48	Scale and fin	1	46	95.8
						2	43	89.6
	Williams Creek spawners	1966	100	91	Otolith and fin	2	83	91.2
	Scully Creek spawners	1966	100	90	Otolith and fin	2	71	78.9
Chum	Skeena River Estuary	1968	50	42	Scale and fin	1	31	73.8
						2	35	83.3
	Bella Bella Area	1968	100	84	Scale and fin	1	82	97.6
						2	64	76.2
	Fraser River	1968	150	145	Scale and fin	1	141	97.2
						2	144	99.3

generally lead to the same estimates of ocean age, the poorest agreement being 78.9% for the samples of Scully Creek spawners. For Babine sockeye, estimates of ocean age from otoliths and fin rays by five readers were generally about the same. Only one agreement rating (86.6%) fell below 90%. Paired estimates are presented in Table 2 for cases in which the estimates of age differed.

Age estimates by the five readers for Babine sockeye disagreed in 30 cases: in 17, older ocean ages were estimated by using otoliths than by using fin rays; in 13, younger ocean ages were estimated. In Scully Creek sockeye, agreement was the lowest: in 7 of the 19 cases of disagreement, older ocean ages were estimated by using otoliths than by using fin rays; in the other 12 cases, younger ocean ages were estimated. Age estimates disagreed for a total of 64 sockeye: in 36, older ocean ages were estimated by using either scales or otoliths than by using fin rays; in 28, younger ocean ages were estimated. It is concluded from these data that there is no bias in the methods of aging; all should yield estimates of similar precision in large samples.

For chum, the scale and fin ray methods also generally lead to the same estimates of total age. Agreement was on the average lower than for sockeye, the poorest agreement being that of 73.8% for the samples of Skeena River chums. In 18 Skeena chum, age estimates by the two readers disagreed: in 14 cases, older ages were estimated by using scales than by using fin rays; in 4 cases, younger ages were estimated. Since some of the Skeena chum fins were removed well above the base, which may have resulted in a loss of the first annual ring on the ray, these data may indicate problems associated

TABLE 2. Comparison of ages[a] of sockeye[b] and chum salmon, determined from fin rays and either scales or otoliths, for instances in which the estimates of age differed.

Species	Location	Reader no.	No. salmon	Age determined from		
				Scales	Otoliths	Fins
Sockeye	Babine Lake spawners	1	5		.3	.2
			1		.2	.3
		2	5		.2	.3
			3		.3	.2
		3	1		.2	.3
		4	8		.3	.2
			3		.2	.3
		5	2		.2	.3
			1		.3	.2
			1		.3	.4
	Skeena River Estuary	1	2	.3		.2
		2	2	.2		.3
			3	.3		.2
	Williams Creek spawners	2	1		.2	.3
			7		.3	.2
	Scully Creek spawners	2	3		.1	.2
			9		.2	.3
			7		.3	.2
Chum	Skeena River Estuary	1	1	0.3		0.1
			7	0.3		0.2
			2	0.3		0.4
			1	0.4		0.3
		2	1	0.2		0.3
			4	0.3		0.2
			1	0.3		0.4
			1	0.4		0.3
	Bella Bella Area	1	2	0.2		0.3
		2	9	0.2		0.3
			8	0.3		0.2
			1	0.3		0.4
			1	0.4		0.3
			1	0.4		0.6
	Fraser River	1	2	0.3		0.2
			2	0.2		0.3
		2	1	0.2		0.3

[a] The European system of age designation (Koo, 1962) is used in this report.
[b] Only ocean annual rings are given.

mostly with the technique of removing the fin, rather than with recognition of the annual ring. Examination of various sections from different locations on a single ray indicated that the innermost annual ring on the section from near the base was not present on sections from further out on the ray (Fig. 1c). Age estimates from these fins may have been biased by being too small.

Age estimates by reader 2 disagreed for 20 Bella Bella chum: in 9 cases older, and in 11 cases younger, ages were estimated by using scales rather than fin rays. There appeared to be no bias in comparative age estimates. In reading the ages of Fraser River chum, the agreement by scales and fin rays was the highest. Age estimates by reader 1 disagreed for 4 chum: in 2 cases older, and in 2 cases younger, ages were estimated by using scales rather than fin rays.

In summary, for both sockeye and chum salmon the pectoral fin ray method generally provided the same estimates of either ocean or total age as the scale or otolith methods. For sockeye, freshwater age could not be determined from the fin ray. Particular care is required in the removal of fins or fin rays from the fish. They must be removed as close as possible to the point of articulation to include the widest part of the fin ray. In the field, it is not necessary to remove the entire fin, only inch-long sections of two or three of the most anterior rays. A clipper, such as surgical bone cutters or pruning shears adapted to remove individual rays, would make the operation relatively simple.

ACKNOWLEDGMENTS

The authors would like to thank Mr Ian Todd of the Department of Fisheries for the collection of fins from the Skeena River estuary, Mr F. C. Withler for his advice and helpful criticisms, Mr R. Cagna for construction of the saw, and Mr G. Atkinson for construction of the ray holder.

REFERENCES

Bilton, H. T., and D. W. Jenkinson. 1968. Comparison of the otolith and scale methods for aging sockeye (*Oncorhynchus nerka*) and chum (*O. keta*) salmon. J. Fish. Res. Bd. Canada 25: 1067–1069.

Bilton, H. T., and W. E. Ricker. 1965. Supplementary checks on the scales of pink salmon (*Oncorhynchus gorbuscha*) and chum salmon (*O. keta*). J. Fish. Res. Bd. Canada 22: 1477–1489.

Boyko, E. G. 1946. Age determination in fishes, based on examination of fin ray sections. Compt. Rend. Acad. Sci. Moscow N.S. 53: 483–484. (Reprinted in 1950 in Progressive Fish-Culturist 12: 47–48.)

Chugunova, N. I. 1959. [Age and growth studies in fish.] A. N. Severtsova [ed.] Akad. Nauk SSSR Otd. Biol. Nauk. Ikhtiol. Komis. Inst. Morfol. Zhivotn. Im.: 1–132. (Publ. for Natl. Sci. Found., Wash., D.C., by Israel Program Sci. Transl., Jerusalem, 1963.)

Clutter, R. I., and L. E. Whitesel. 1956. Collection and interpretation of sockeye salmon scales. Intern. Pacific Salmon Fish. Comm. Bull. 9. 159 p.

Cuerrier, Jean-Paul. 1951. The use of pectoral fin rays for determining age of sturgeon and other species of fish. Canadian Fish Culturist 11: 10–17.

Koo, Ted S. Y. 1962. Age designation in salmon, p. 37–48. *In* T. S. Y. Koo [ed.] Studies of Alaska red salmon. University of Washington Press, Seattle, Wash.

Scidmore, W. J., and A. W. Glass. 1953. Use of pectoral fin rays to determine age of the white sucker. Progressive Fish-Culturist 15: 114–115.

Size Changes, Mortality, and Equilibrium Yields in an Exploited Stock of American Plaice (*Hippoglossoides platessoides*)

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Records from research surveys and commercial landings for American plaice from the Magdalen Shallows, or the southwestern Gulf of St. Lawrence, showed decreases in percentage of old, large fish and in catch per unit of effort from 1955 to 1962. The changes in age composition were clearly relatable to the fishery, which was mainly by otter trawl. Ricker models indicated that wastage of deck-exposed subcommercial plaice is currently a greater mortality factor than predation by cod. Increased landings in particular years were associated with successful year-classes. Other factors affecting landings were annual differences in fleet dispersal, which were related to movements of cod, a cohabiting species. No clear decrease or increase in absolute recruitment of plaice was demonstratable because quantitative comparisons of research surveys by different vessels using different gears and with different skippers, would be misleading.

Mortality estimates of adult plaice by three methods showed agreement, indicating that instantaneous rate of natural mortality was between 0.09 and 0.13. The instantaneous rate of fishing for 1957–62 was 0.46. Increasing mesh-size would reduce cod catches and have little effect in conserving plaice, but marketing small fish would increase plaice landings. Voluntary release of small plaice promptly on capture would help maintain the stock, as would reduction in numbers of large cod, the main predator of small plaice. Increased numbers of small cod since 1959, as reported by other workers, could result in greater food competition with small plaice, effectively reinforcing the ecological dominance of cod over plaice.

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INTRODUCTION

The natural history and ecology of American plaice, and predation by large cod, for the Magdalen Shallows (Fig. 1) were reported earlier (Powles, 1965). This paper describes the history of the fishery, changes in the stock structure, and factors affecting abundance and yield of plaice.

In a research survey by beam-trawl, Huntsman (1918) recorded American plaice as extremely numerous in the Magdalen Shallows, although virtually none were landed. Those landed were taken incidentally by hook and line with cod. However, an otter-trawl fishery began in 1947, and Danish seiners were added to the fleet in 1958. By 1964 over 150 offshore vessels (trawlers and seiners) fished for plaice in the Magdalen Shallows.

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Next to Atlantic cod, plaice appears to be the second most abundant groundfish species inside the 100-fath contour of the area. Because their distributions are so similar (Fig. 2), the cod and plaice fisheries are closely linked.

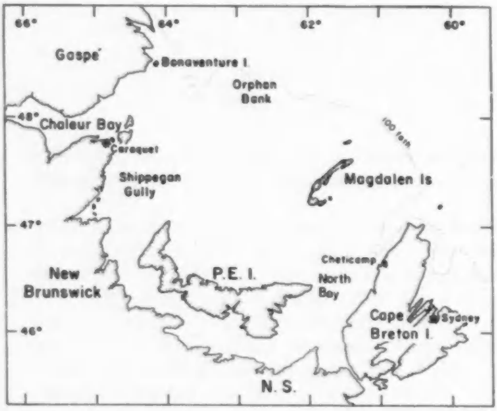

FIG. 1. The Magdalen Shallows, with localities mentioned in text.

Documentation of the plaice fishery became important since a gradual decline in the numbers of large cod (Jean, 1963; Paloheimo and Kohler, 1968) caused smaller vessels at times to rely mainly on catches of American plaice.

GENERAL METHODS

The Magdalen Shallows are designated as Division 4T (Fig. 2) by the International Commission for the Northwest Atlantic Fisheries, and in this paper the areas and the various parts of it are on this basis.

Information on commercial fleet distribution and fishing effort was obtained from log records kept by captains of fishing vessels in cooperation with the Fisheries Research Board of Canada. These records were used primarily in the analyses of the commercial landings, and were complemented by shore sampling and research-vessel surveys which form the main basis of this study.

Research surveys were carried out in different seasons during 1957–62 as described by Powles (1965). Three research trawlers were used: the 65-ft *J. J. Cowie* (summers, 1957–58), the 84-ft *Harengus* (summers, 1959–62), the 177-ft *A. T. Cameron* (winters, 1960–61). All vessels used otter trawls and 1.5-inch (38-mm, stretched) mesh covers or liners on the codends of the nets.

Quantitative estimates of plaice discarded at sea were obtained by (1) placing observers on commercial vessels; (2) comparing research-vessel catches with commercial landings; and (3) estimating amounts of discards from cooperating skippers keeping specially designed log records of their catches.

Prior to 1958, flatfish (excepting halibut) from the area were not sorted by species at plants; thus landing statistics were only available as unspecified flatfish. However, 1957 research surveys indicated that 90% of the flatfish in the northern Magdelan Shallows were American plaice, and about 80% of those in the southern half. Knowing the seasonal distribution of the fleet from log records, appropriate adjustments were made to the "mixed flounder" category to convert it to pure plaice. The landings shown in Fig. 3 closely approximate the true landings of American plaice. Greysole (*Glyptocephalus cynoglossus*) and winter flounder (*Pseudopleuronectes americanus*)

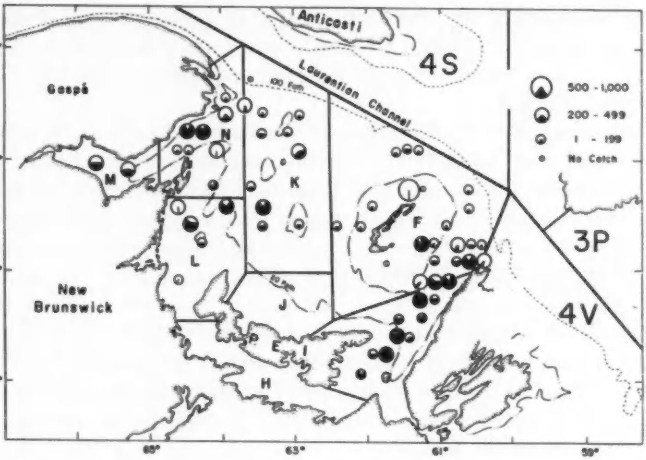
Anticosti
4S
Laurentian Channel
100 Fm
Gaspé
N
K
M
L
F
3P
4V
New Brunswick
J
P E I
H
G
500 - 1,000
200 - 499
1 - 199
No Catch
49°
48°
47°
46°
65°
63°
61°
59°

FIG. 2. Ratios for plaice (*black*) and cod (*white*) in catches in the Magdalen Shallows from ½-hr otter-trawl sets by research vessel in 1960 and 1961.

were the other flatfish landed commercially. However, research surveys showed that the former species occurred in deep water, and that the winter flounder was confined to shallow waters in localized areas.

THE FISHERY AND STOCK

In 1935 landings of only 300,000 lb were made from the area, all from the northern part. By 1947, landings were still only in the vicinity of 800,000 lb (Fig. 3). In 1948, landings began to increase markedly, and by 1955 reached a peak of 26 million lb. From then to 1965, there were several fluctuations above and below a mean of 22 million lb per annum. These are given special

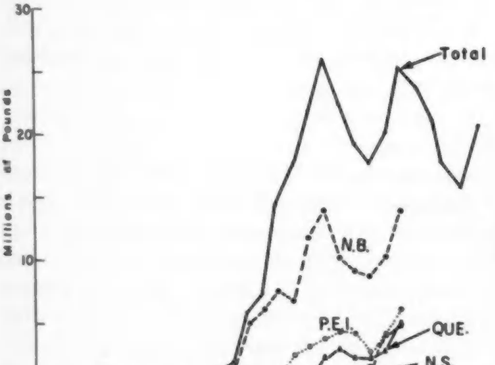

FIG. 3. Annual plaice landings from the Magdalen Shallows; 1937–46 landings were by hook and line and those for 1947–67 by otter trawl, Danish seine, and hook and line, combined. From 1948–60, landings are shown separately by provinces.

consideration later on. By 1965, practically all landings were made by otter trawlers. Landings by hook and line reached only 500,000 lb per annum, catches which were made by small inshore hand-lining boats and a few which set line trawls for cod.

The commercial study fleet, over 80 vessels from northern New Brunswick, ranged in length from 45 to 110 ft. The several classes included: the "Chaleurs" and Danish seiners, both under 60 ft long; the "Gloucesters" and name vessels, all over 60 ft. Catches of Chaleur vessels had a higher percentage of plaice than the larger vessels (40% vs. 25%), but larger vessels together landed more plaice. Danish seiners took about 60% plaice per trip in the northern half of the Shallows.

During 1947–52 the commercial fleet fished in waters near Bonaventure Island south and west from Chaleur Bay and Shippegan Gully. From 1953 onwards, however, the larger vessels fished throughout the Shallows. In 1960 and 1961 vessels moved out of the Shallows in their quest for cod, and only smaller vessels fished the area.

Fleet dispersion (Mousette et al., 1965) was related to the seasonal distribution of cod in the area (Jean, 1963) and to some extent, that of plaice. In early spring cod move north from eastern Cape Breton into the Shallows, a portion of the stock spreading west and the rest continuing north. By summer they may reach the northern portions of the Shallows and extend north and west of Anticosti Island. By late fall and early winter the cod return south. In late winter, a fishery by large offshore vessels generally occurs along the Laurentian Channel, in waters east of Cape Breton.

There are two groups comprising the Shallows plaice stock (Powles, 1965). These two groups do not mix. One occurs in the northern and the other in the southern sector of the Shallows. Plaice, unlike cod, remain within the Shallows in summer but move into deep water along the Laurentian Channel in winter. In early spring both groups move inshore again, and converge with cod as the latter move north and west into the Gulf. Since plaice inhabit the same depths and temperatures as cod, both species are subjected to much the same fishing effort in summer. The cod are fished in late winter and early spring before migrating to the Shallows, but the plaice stock is not exploited in winter because of fast or drifting ice.

MESH SELECTION AND DISCARDS

The codends used by most fishermen from 1947 to 1958 were of 3-inch (76-mm) mesh manila, but by 1959 most had converted to 4.5-inch mesh. By 1960 almost all vessels had switched to 4.5-inch synthetic codends.

For 3-inch mesh, 50% of fish aged 2.8 yr (11 cm long) were retained; for 4.5-inch mesh the corresponding ages were 6.2 years (25 cm) for males and 6.0 years (25 cm also) for females. The 5.5-inch synthetics mesh retained 50% males age 8 years, and 50% females age 7.3 years (30 cm) (Fig. 4).

Selection studies of Danish seine nets indicated higher retention lengths than for otter trawlers of the same mesh (Powles, MS, 1964). The reasons

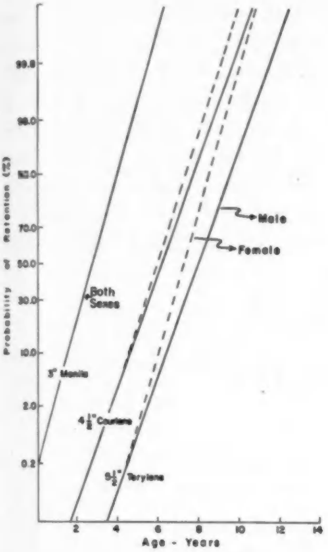

FIG. 4. Selection properties of three sizes of mesh with respect to American plaice according to age and sex. Based on 3789 measurements, May–July.

for this are unknown, but similar results for European flatfish were obtained by Margetts (1949) for British trawler and seiner selection comparisons.

RELATION BETWEEN CATCH STATISTICS, EFFORT, AND LANDINGS

Commercial catch statistics were examined in relation to the biology of plaice and the history of the fishery. Total landings, catch per unit effort (catch per week), and total effort were examined first. A more detailed examination of catch per trip per unit area was then made for that segment of the fleet possessing detailed log records. Considerable caution was required in interpretation because of the close relationship between cod and plaice fishing. Usually cod and plaice overlap in distribution, so catch-per-trip estimates were not biased by the nature (whether plaice or cod) of the trips in summer, when the bulk of plaice were landed.

Figure 5A shows that from 1949 to 1955 there was a regular rise in landings, due to the introduction of otter trawlers to the area. This rise ended, with a peak of 26 million lb, in 1955. From 1956 to 1958 landings fell to a low of 18 million lb in spite of an increase in effort for those years (Fig. 5C). In 1959 and 1960 there followed a slight rise, and from 1961 to 1964 another drop in landings, whereas effort rose slightly for 1959–61. After 1961 it was impossible to estimate effort on plaice by vessel class. Landings in 1965, however, rose to 20 million lb. The decline in landings after the first peak in 1955 paralleled a decrease in catch per trip for larger vessels and thus represents a true decrease in abundance. The decline in landings after the second peak of 1960, however, was due to two other factors: (1) late commencement of fishing

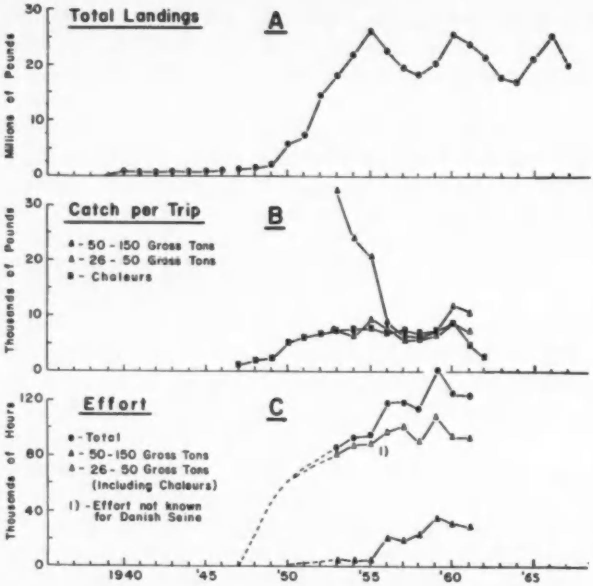

FIG. 5. (A) Annual plaice landings from the Magdalen Shallows, all vessels, 1940–67. (B) Catches per trip (catches per week) by vessel type. (C) Total effort broken down into two vessel size categories.

(due to ice) and (2) "cod-seeking." It is almost impossible to separate these factors. In 1960–61 apparent decreases in effort were due to a general exodus of the larger boats from their usual grounds to the Seven Islands region (4S), where substantial catches of cod were made. Nevertheless, catch per trip of plaice increased in 1960 as a result of: (1) the smaller vessels specifically seeking plaice, combined with (2) an atypical offshore concentration of plaice. In that year plaice concentrated (inexplicably) in the Orphan Bank region, and fishermen were quick to exploit them.

The catch per trip of Chaleur class vessels is a good index of plaice abundance because the boats fish on regular grounds close to home. The effort of Chaleur vessels went up steadily from 1953 to 1957, then levelled off. Catch per trip rose up to 1960, then dropped. The Chaleur's catch-per-trip level by 1962 dropped to less than one-half that in 1960. Since sizes were also smaller by 1959–60, vessels had to land more young fish to maintain profitable trip-poundage. It is quite evident that catch per unit effort decreased after 1960 in the areas fished by these vessels.

More detailed records from a small segment of the commercial fleet allowed a break down of catch-per-trip data by unit area for 1955–60 (Fig. 6). This period showed marked changes within the stock brought about by the fishery. Detailed records do not extend back earlier than 1955, but early descriptions of the fishery state that in 1953, 1954, and 1955, unusually high numbers of post-spawning plaice were taken in May and June on shallow banks north of the Magdalen Islands (4Tf). This is confirmed for 1955 (Fig. 6B, broken line and asterisk) by statistics of the catches per trip. The catch-per-trip records for unit areas 4Tl, m, and n, however, showed no peak in 1955, but remained steady throughout the years 1955–60. The increased fishing of large fish in unit area 4Tf augmented landings because that stock of plaice had been hitherto relatively unexploited. Though small, Chaleur class vessels shared in the record catches of 1954–55 in 4Tf.

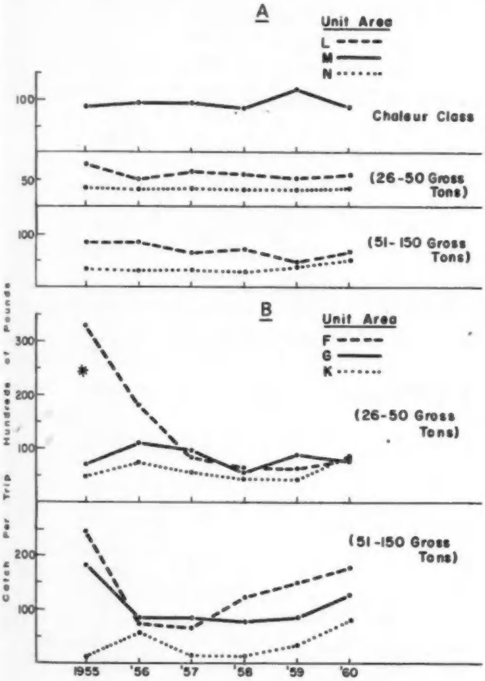

FIG. 6. Catches of plaice per trip (catches per week) for the Shallows by vessel class, unit area (Fig. 2), and year. The asterisk (*) in panel B represents Chaleur class catch per trip of 1955, the only year in which they fished unit area F.

Another factor augmenting landings in particular years was the contribution of strong year-classes to the fishery (Fig. 7). In 1955, the year of peak landings and relatively high catch per trip, the strong 1943 year-class made up 20% by number of the landed catch. Since fish of this year-class were 45–50 cm long at the time, they contributed heavily to total weight landed. From 1956 to 1958, the years of apparent decline in catch per trip, no large year-class except that of 1950 was present, and even this had just been recruited. This year-class was large (30% by number from 1957 to 1959) but it did not contribute that much in terms of weight, since it was not yet completely recruited to the fishery.

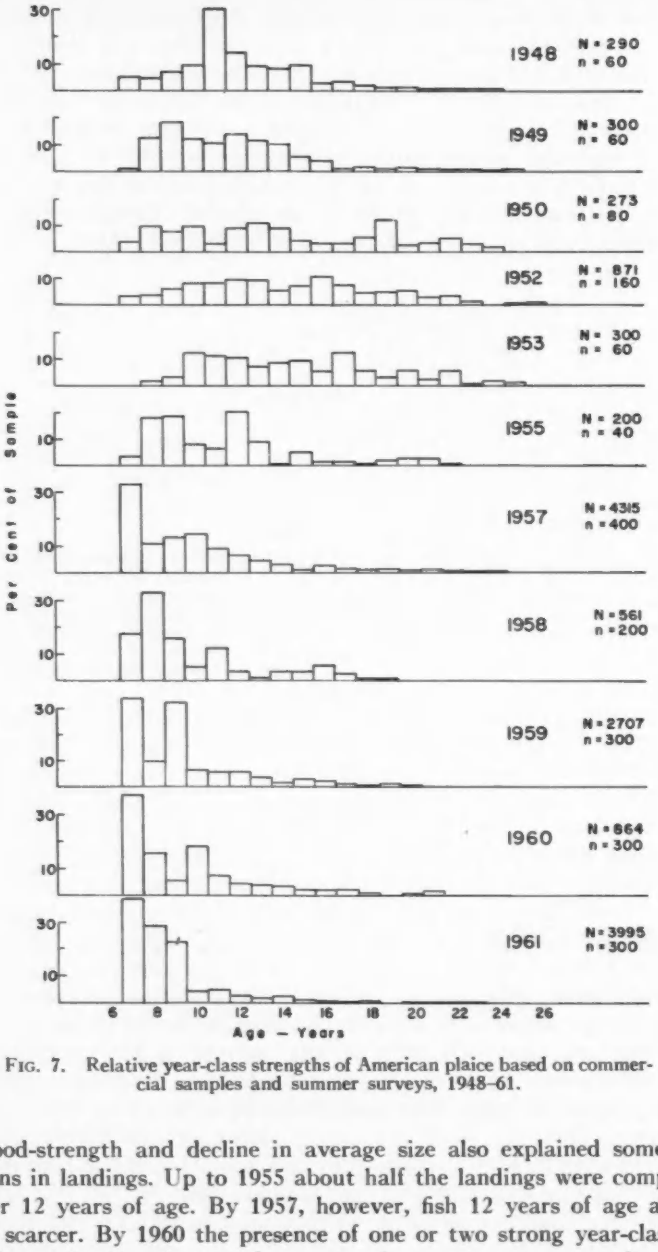

FIG. 7. Relative year-class strengths of American plaice based on commercial samples and summer surveys, 1948–61.

Brood-strength and decline in average size also explained some of the variations in landings. Up to 1955 about half the landings were composed of fish over 12 years of age. By 1957, however, fish 12 years of age and over became scarcer. By 1960 the presence of one or two strong year-classes had more effect on landings because fewer year-classes were present on the grounds.

The effect of the 1950 year-class in 1960 was greater than that of the 1943 year-class in 1955 (it could have been a larger year-class). Brood strengths did not in themselves account for all peaks or dips in landings and catch per unit of effort, though in most instances their effects did contribute to the observed trends. Relative effects of brood strength on commercial landings will increase in future as the number of year-classes is further reduced.

Since 1950 reduced landings or catch per unit of effort may be explained primarily in terms of: (1) reduction in numbers of large fish, particularly in 1954–55; (2) atypical areal changes in distribution, 1960 only (Powles, 1965); and (3) increased plaice-seeking tendency in years that codfishing was poor (1960–61). These factors go a long way in explaining variations in the estimates of abundance. In terms of decrease in landings, reduction in the numbers of large fish was the most important single factor. The actual abundance of younger fish has remained high enough to support the fishery in later years.

CHANGES IN SIZES CAUGHT AND LANDED

In Hook-and-Line Fishery

Apart from early samples from rocky grounds, which might tend to be unrepresentative of the plaice fishery, there are one record of a complete catch and two records of landings of American plaice taken in the early hook-and-line fishery on otter trawl grounds from 1946 to 1949. These suggested that only large fish were caught and none discarded. Since total landings per annum of plaice even up to 1947 did not exceed 700,000 lb, and since the early fishery removed only large fish, there was evidently little effect on size distribution.

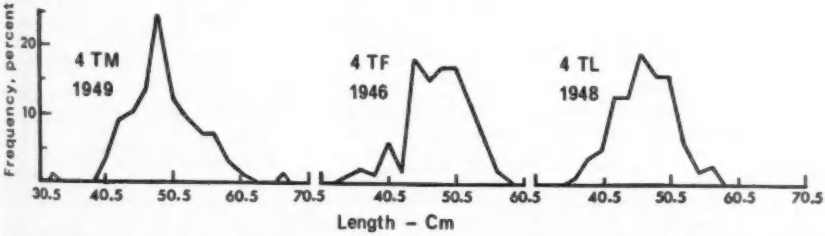

FIG. 8. Size compositions of an early catch (4TM) and of two landed samples (4TF and 4TL) of American plaice by hook and line.

In Trawl and Seine Fisheries

In these fisheries percentages of large fish in landings generally declined from 1952 to 1963 (Fig. 9). Rate of change varied somewhat by area, but was difficult to assess quantitatively. Noticeable changes occurred in the Shippegan Gully (4Tl), Sidney Bight (4Va), and Cape Breton (4Tg) areas, with changes in mean size over the years of about 12 cm. The mean size in Chaleur Bay (4Tm) decreased by only 7 cm, but no otter trawl catches are available for years before 1955.

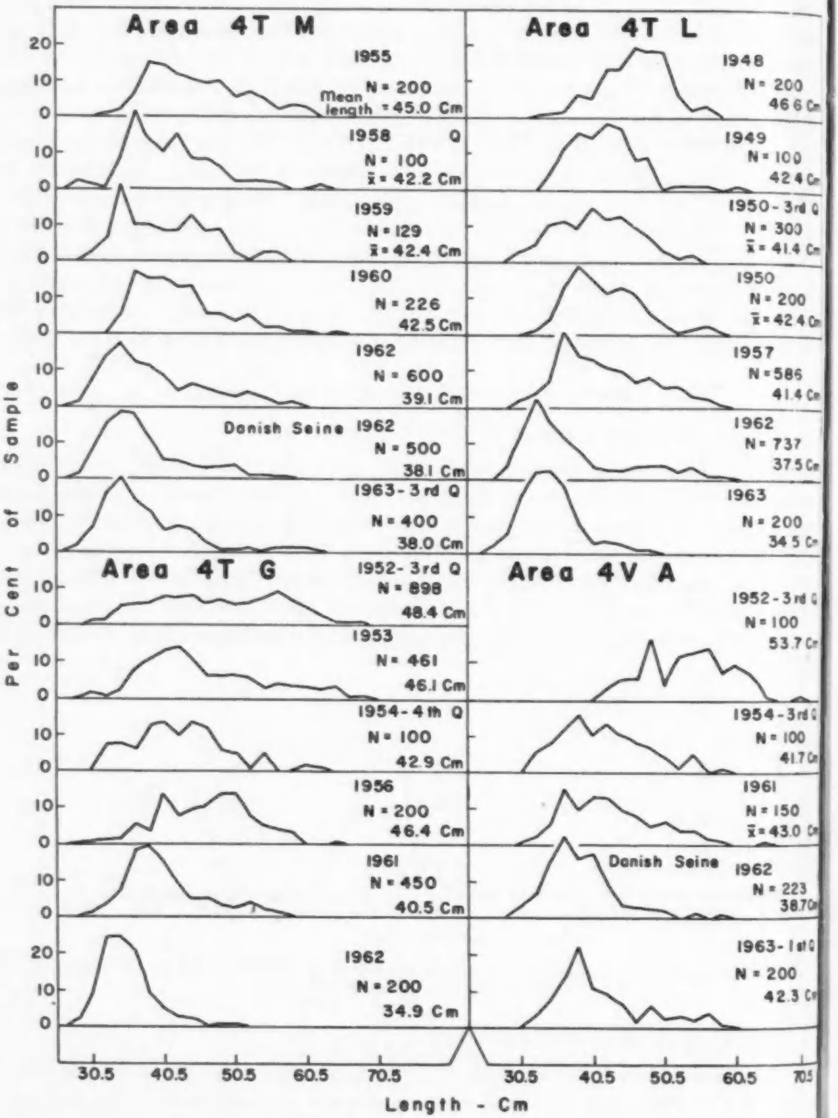

FIG. 9. Length frequencies of American plaice in landings by otter trawl and Danish seine fisheries from Chaleur Bay (4TM), Shippegan Gully (4TL), and North Bay (4TG), for the 2nd quarter unless otherwise indicated. An adjacent area, 4VA, is also included.

As sizes of fish decreased, smaller fish became acceptable for market. The trend is most noticeable from Shippegan Gully samples. During 1948–50 no fish under 30 cm were accepted. From 1962 on, the proportion of plaice under 30 cm gradually increased in the catches.

Further data supporting changes in size distribution were obtained from research surveys between 1957 and 1962 (Fig. 10). These surveys showed that plaice over 25 years of age were common in 1957, but by 1962 the oldest females were 19. Males showed similar decreases.

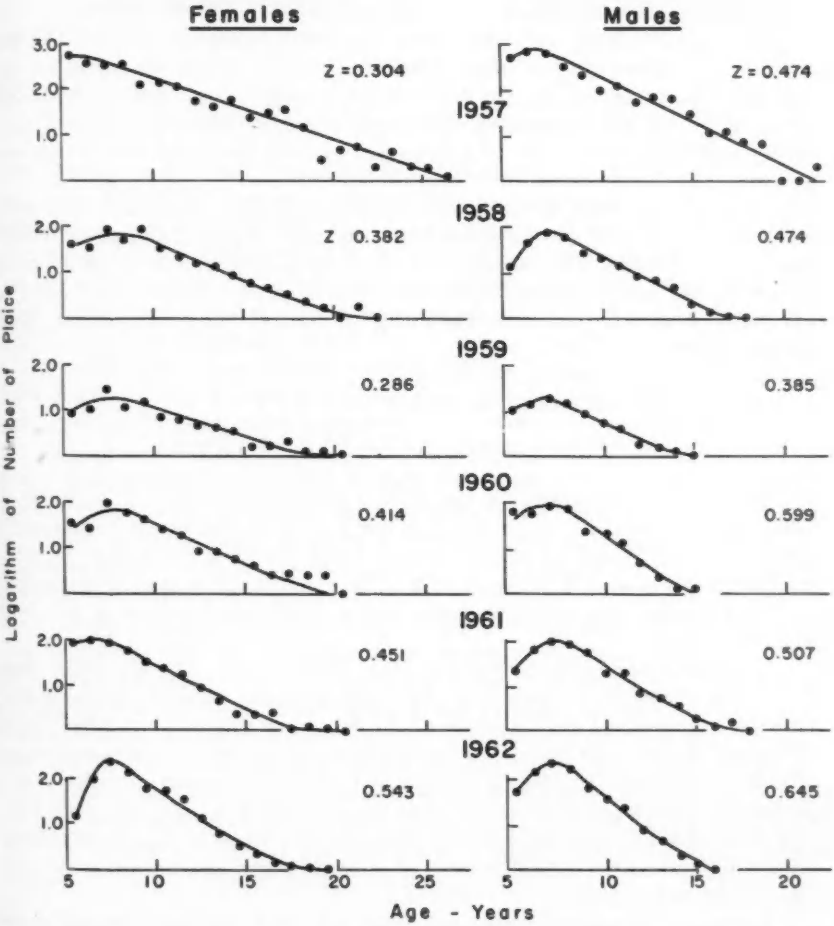

FIG. 10. Catch curves for American plaice obtained from research surveys of the Shallows during 1957–62. Total instantaneous mortality estimates (Z) are shown to the right of each curve.

MORTALITY ESTIMATES

From Age Composition of the Fully Recruited Stock

The earliest mortality estimate for American plaice in the study area was made in 1917 (from an unsexed sample) by Huntsman (1918) when the species was virtually unfished. Total mortality estimates were also computed by the author just after the onset of otter trawling in 1950, and further estimates were made from research-vessel data for 1957 to 1962. The evaluations of total mortality in 1917 and 1950 represent close approximations to the natural mortality, since the population was close to the virgin state, particularly in 1917.

Huntsman (1918) estimated that the total mortality was 12.5% per annum (instantaneous rate of $Z = 0.14$). This conclusion was based on age composition ratios for several tows made by small-mesh beam trawl. His value of 12.5% may represent the current value of adult natural mortality if environmental conditions have not changed over the years, and if methods of ageing are kept similar.

A similar estimate of total mortality was made, by presumably the same method, from a 1950 sample collected in the same area and season as Huntsman's 1917 sample. This sample was taken by commercial otter trawl using 3-inch manila codend. Considering only plaice of age 9 years and older to eliminate possible errors from discarding at sea, the age-composition ratios for successive ages were:

$$\frac{23 + 14 + 20 + 16 + 7 + 8 + 4 + 0 + 2 + 2}{17 + 23 + 14 + 20 + 16 + 7 + 8 + 4 + 0 + 2} = 0.87$$

Hence total annual mortality was 13% (instantaneous rate $Z = 0.13$), a value close to that calculated by Huntsman ($Z = 0.14$) for 1917. A slightly different method (Robson and Chapman, 1961) gave a Z value of 0.26 (95% confidence limits were 0.265 ± 0.092). This value is not as heavily dependent on the younger age-groups, includes fishing mortality, and so is probably the more reliable.

Tagging experiments carried out from 1958 to 1961 (Powles, 1965) show that plaice of ages over 9 years are uniformly catchable. Sizes of returns during 1958–61 were plotted against those of the fish when tagged (Fig. 11). Within the length range 35–60 cm, which represents plaice aged 9–21 years, recaptures of plaice from both Shippegan Gully and Chaleur Bay were in uniform proportion with the lengths of the fish when tagged. This is good evidence that the ages 9–21 are uniformly susceptible to capture by otter trawl. Furthermore, these fish followed the typical pattern of seasonal migration, so that movement was not limited.

Survival estimates of young fish from age compositions of young plaice taken in research surveys of the study area during 1957–62 showed so much variation as to be only inconclusive. Factors such as net selection and varying

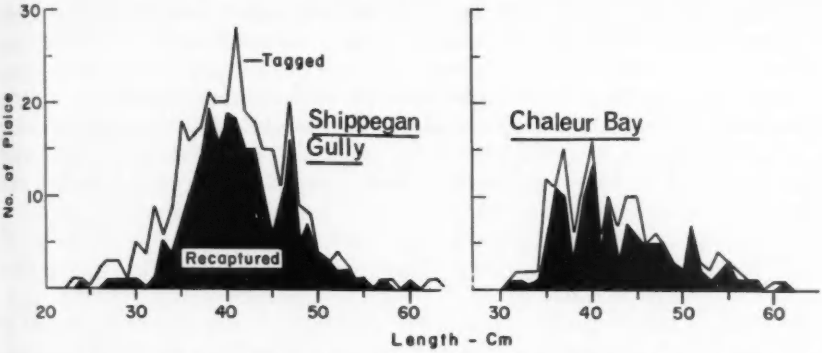

FIG. 11. Size compositions of recaptures (1958–61) of two lots of tagged American plaice in relation to those of the fish when tagged (1958–59).

brood strengths resulted in non-uniform catches. Changes in vessels, skippers, and gear added to the complexities involved in following the absolute reduction of particular broods. The surveys indicated that natural mortality of small plaice was greater than for adults because of predation by cod. Cod was the main predator, selecting only those plaice under 30 cm long. Instantaneous natural mortality due to cod grazing alone in 1961 has been estimated at 0.12–0.20 (Powles, 1965).

Mortality rate estimates of American plaice (sexes combined) were also made from research survey data. Only summer data were selected, at which time size distributions are uniform.

The age-compositions from research surveys of 1957–62 (sexes combined) gave a total annual mortality rate of 40% ($Z = 0.50$). Over the years there was a gradual increase in the slope of the right limb, and by 1962 the slope indicated a Z value of 0.60. Later research surveys in 1962–64 showed that the instantaneous total mortality rate for American plaice was of the same magnitude (Powles, MS, 1964). For purposes of the models, catch curves were broken down by sex (Fig. 10). For the period 1957–62 the total mortality (Z) averaged 0.4 for females and 0.5 for males, each for fish 8 years and older. The 1962 data, which are really more representative of the 1962–66 period, gave a value of 0.54 for females and 0.65 for males. A fairly drastic change in age structure is evident in both the males and females from 1957 to 1962. In 1957 females over age 25 are well represented in the catches, whereas by 1962 no females over age 20 show up. The same general trend is apparent among male plaice.

Figure 5C shows there was an increase in the rate of fishing from 1957 to 1961. Heavy fishing is known to produce concavity in the catch curves (Ricker, 1958), and this is apparent in Fig. 10.

FROM TAGGING EXPERIMENT

In the tagging experiments, conducted mainly for information of growth and movements (Powles, 1965), 1732 fish were marked from 1958 to 1960. Only data on fish tagged in early summer and late spring were selected for mortality studies, since later in the season high surface temperatures result in tagging mortality. The estimates of fishing rate and natural mortality are therefore probably reliable. To assess inherent sources of error, comparisons of natural mortality were made from primitive age compositions, and independent estimates of total mortality were available from research surveys.

Four tagging lots (Fig. 12) form the basis of this analysis. Lots A–C were released in known commercial fishing areas, nearly all fish being over 8 years of age and therefore fully vulnerable to the fishery. Lot D fish were large, mature plaice, representing segregated groups occurring on sandy, shallow bands in spring and early summer.

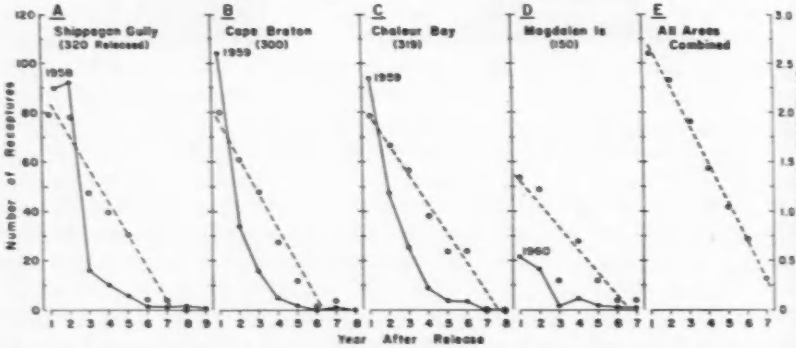

FIG. 12. (A–D) Numbers of tagged plaice recaptured in successive years after release in four areas (*solid lines*), and logarithms of numbers recaptured (*broken lines*). (E) Logarithms of total recaptures, all areas combined.

Figure 12A–D shows that fish in lots A, B, and C were returned in similar proportions by year, except that lot A had very high recaptures in 1959. This exception was probably due to the fact that Danish seiners added to the fleet in that year augmented plaice landings. No attempt was made to adjust for fishing effort because of the lack of information on total effort for each tagging area. Returns from lot D (Magdalen Islands), although not great, nevertheless indicated that long-term survival was similar to that of other lots. The total mortality of these older plaice is therefore not markedly higher than that of younger fish, an important point in the consideration of models to be discussed later.

When all four tagging lots were combined and the logarithms of recaptures plotted in successive years after release (Fig. 12E), a remarkably uniform picture of returns was obtained, representative of the area as a whole. The slope was equivalent to a survival rate of 43.3% per annum, corresponding to a total instantaneous mortality rate of $Z = 0.84$.

Beverton and Holt (1957) and Ricker (1958) outlined the many sources of error which may affect estimates of mortality from tagging experiments

Ricker divided these errors into three types: A, B, and C. Among group A errors, which affect estimates of fishing rate but not survival, the only concern was incomplete reporting of tags. Since no special procedures were carried out to test this factor, it may be assumed only that about 1 in 20, as a guess, was not reported. This would result in an estimate of the fishing rate which is about 5% too low.

Concerning type B errors, the fish were tagged in compact groups in commercial fishing areas, and were not spread out randomly within the area. Since there is a tendency for fish to spread out in successive years, this is tantamount to a progressive emigration to less heavily fished areas each year. Such a factor would lead to too high a value for total mortality, although the estimate of fishing rate is unaffected. Comparisons with results of computing survival from age-composition ratios (Table 1) support this hypothesis. The total instantaneous mortality rates from age compositions are both much smaller than that arrived at from tagging data. Furthermore, the estimate of apparent natural mortality obtained from the tagging data is unreasonably high for such a long-lived species as American plaice.

Since an adjustment of year one returns from lot A (Shippegan Gully) fish prior to incorporation in Fig. 12A did not cause the point to fall much above or below the point expected, it is unlikely that type C errors (marked fish more or less vulnerable in the first year) were involved. It is possible, of course, that nonrandom distribution of marked fish in the first year was balanced by some sort of nonrandom distribution of fishing effort. In any event, type C errors are not serious when good recovery data are available for several successive years after tagging.

Paloheimo's (1958) method was also used to obtain an estimate of natural mortality from the tagging data. The method is applicable when little or no data are available on effort or catch related to tag returns.

For analysis of the tagging data a quarter of the year was selected as the unit of time. By using the formulae

$$N_v + 1 = N_v e^{-(F_v+M_v)}$$

and

$$F_v = \frac{n_v}{N_v} \div \frac{(1 - \frac{n_v}{N_v} + M_v)}{2}$$

where N_v, F_v, and M_v are the number of returns, the instantaneous fishing mortality, and the instantaneous natural mortality respectively, during the course of vth interval, Table 2 was constructed. Corresponding to each assumed rate of the natural mortality, the Table shows estimated population size at the beginning of the quarter and the instantaneous fishing mortality rates during each quarter. The quarterly rates were then summarized to give the total instantaneous fishing mortality rate. The results for the four lots

TABLE 1. Estimates by various methods and best estimates of parameters in mortality of fully vulnerable plaice, based on available age composition and tagging data. (Asterisk (*) denotes "apparent" rate.)

	Source	Survival rate (S)	Rate of total mortality (Z)	Rate of fishing (F)	Rate of natural mortality (M)
		By all methods			
1917	Primitive catch curve	0.87	0.13	Nil	0.13
1950	Catch curve	0.74	0.26	Low	0.26
1957–62	Research cruises	0.61	0.50		
1955–62	Commercial samples	0.60	0.51		
1962–66	Research cruises	0.50	0.60		
1958–66	Tagging	0.43	0.84*	0.46	0.37*
1959–60	Tagging	–			0.11–0.16
		Best estimates			
Primitive catch curve (sexed)					Males: 0.17 Females: 0.13 Combined: 0.14
1957–62	Research cruises (0.50–0.14)			0.36	
1955–62	Commercial (0.51–0.14)			0.37	
1962–66	Research cruises (0.60–0.14)			0.46	
1958–66	Tagging (lots A–D)			0.46	

TABLE 2. Estimates of total instantaneous fishing mortality (F) and related computations for three assumed rates of natural mortality (M).

Quarter of year	Calculated no. of tagged plaice extant			No. of returns	Loss for natural mortality			Estimated fishing mortality (F)		
M =	0.05	0.16	0.29		0.05	0.16	0.29	0.05	0.16	0.29
						1958, Lot 1				
2	320	320	320	36	3	8	22	0.120	0.122	0.124
3	281	276	262	45	4	14	17	0.175	0.184	0.196
4	232	217	200	9	1	8	14	0.040	0.042	0.048
								0.335	0.348	0.368
						1959, Lot 2				
2	300	300	300	85	1	10	15	0.332	0.337	0.344
3	214	205	200	10	2	7	12	0.048	0.050	0.053
4	202	188	178	7	3	7	12	0.036	0.038	0.042
								0.416	0.425	0.439
						1959, Lot 3				
2	319	319	319	17	4	13	22	0.055	0.056	0.057
3	298	289	280	55	2	10	17	0.205	0.215	0.228
4	241	224	208	22	3	8	14	0.098	0.105	0.116
								0.358	0.376	0.381
						1960, Lot 4				
2	150	150	150	14	2	6	10	0.098	0.100	0.101
3	134	130	126	4	2	1	9	0.031	0.032	0.036
4	128	125	113	4	2	5	9	0.032	0.033	0.037
								0.161	0.165	0.174

are plotted in Fig. 13, together with the independent estimates of total mortality obtained from age composition ratios, indicated by vertical lines. On the basis of these ratios, the instantaneous natural mortality rates were: 0.11 for lot D (Magdalen Islands) fish and between 0.09 (Shippegan) and 0.13 (Cape Breton) for fish in lots A–C.

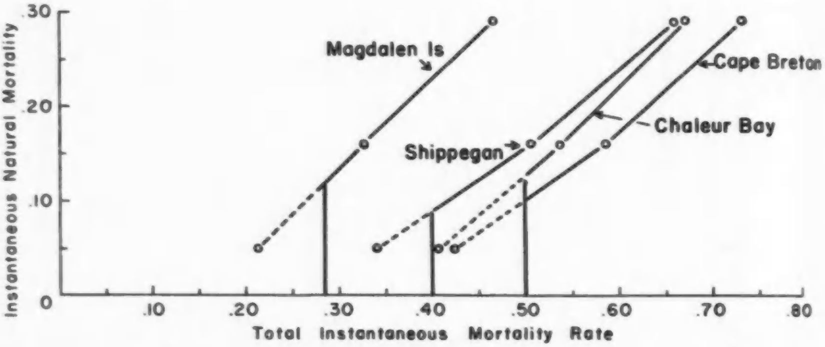

FIG. 13. Estimates of total mortality rates of American plaice on basis of three assumed rates of natural mortality for four tagging lots. Vertical lines indicate the estimates of total mortality obtained from age-composition ratios.

Years were divided into two periods for estimates of rate of fishing. During the earlier phase of the fishery, 1957–62, the instantaneous rate of fishing appeared to be about 0.36. For later years, 1962–66, the rate of fishing increased to 0.46. The natural mortality rate of plaice fully vulnerable to the fishery apparently remained constant through the years.

WASTAGE AT SEA

ON OTTER TRAWLERS AND DANISH SEINERS

Estimates of quantities and sizes of plaice discarded at sea by various gears are essential requirements for calculating the effects of fishing on the stock. In the present study, estimates of sizes and amounts caught and discarded by commercial vessels were recorded and compared to theoretical discard estimates computed from research-vessel survey hauls and mesh-selection data.

Figure 14 shows that there were considerable differences in size distributions in both the catches and discards of otter trawlers and Danish seiners fishing the same grounds. In both northern and southern sectors (4Tm and 4Tg), catches made by Danish seiners contained proportionately fewer small fish and more large ones than those by otter trawlers. Consequently, discards by the Danish seiner were much lower than by the otter trawler. Discards by number ranged from 85% (4Tn) to 54% (4Tm) for otter trawler, and from

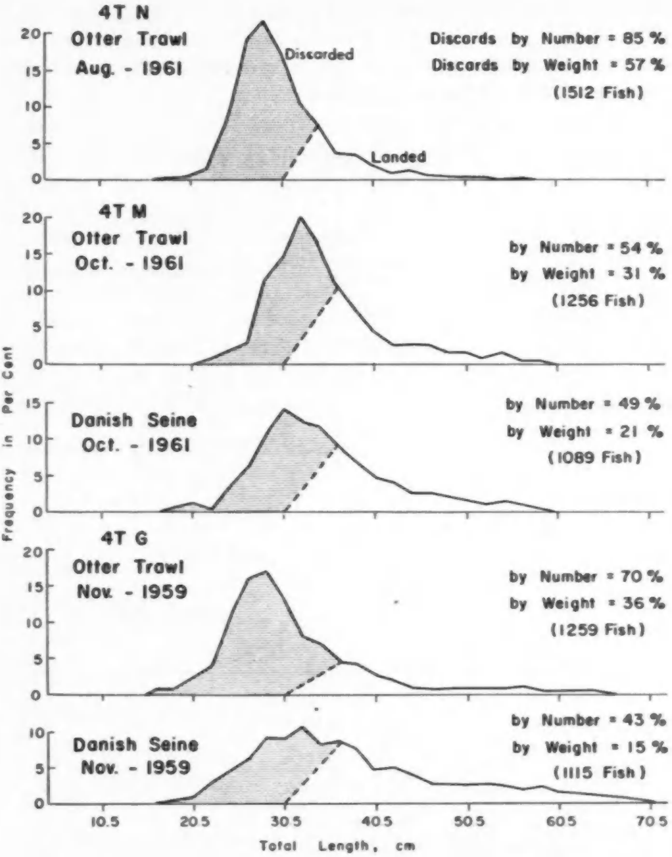

FIG. 14. Size compositions of American plaice caught and landed by Danish seiner and otter trawler within various areas; in 4TM and 4TG the two types of vessel were fishing the same grounds. All vessels used 4.5-inch codends. Discards are shown stippled.

49 to 43% for Danish seiner. The smaller discards and larger sizes taken by the Danish seiner are in accord with the differences in selection found for the two types of gear (Margetts, 1949; and others).

SIZE DIFFERENCES BY YEAR, AREA, AND SEASON

Discards vary with season, year, and area. In the northern area 4Tn, there were proportionately more small plaice and fewer large ones in research catches than in 4Tm, l, or f from June to October (Fig. 15). For this reason, wastage was highest in 4Tn. In Chaleur Bay (4Tm) the proportion of large plaice in catches was, and appears to have remained, fairly high. Although

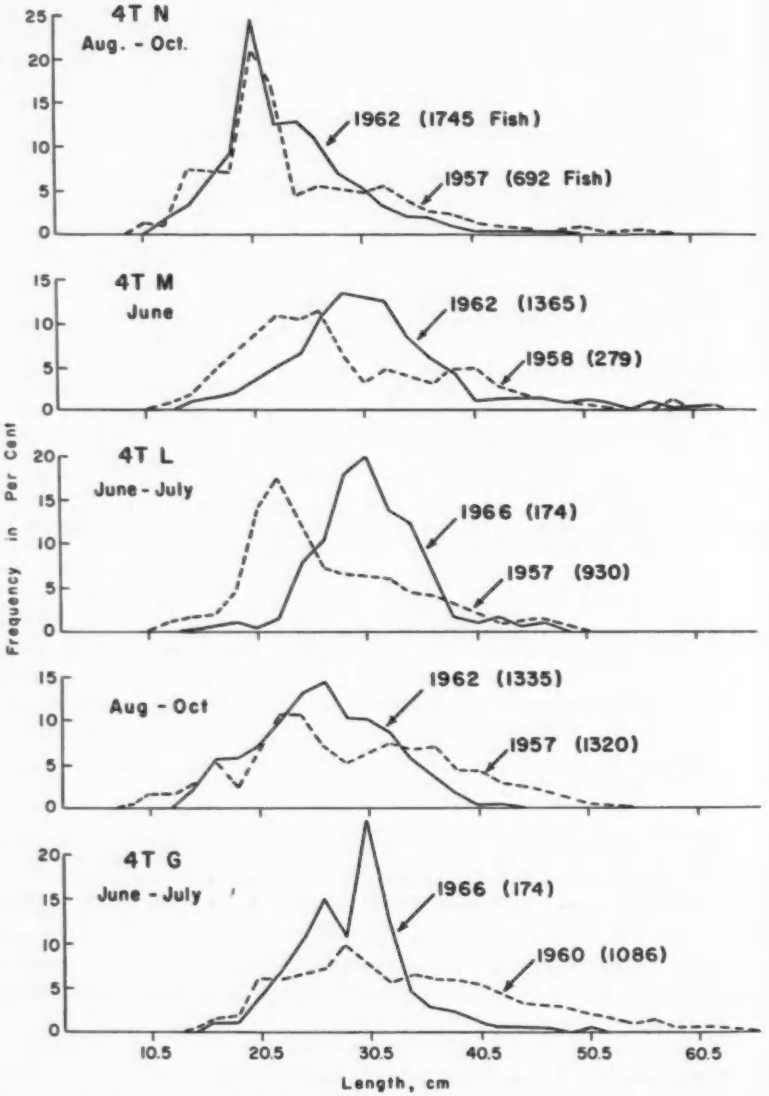

FIG. 15. Size compositions of catches of American plaice in early and late years of research surveys by unit areas.

plaice were abundant in Shippegan Gully, sizes were not particularly large as far back as 1957. Wastage was consequently still high in 1961. North Bay (4Tg) and 4Tm consistently appear to have supported more of the largest sizes of plaice (over 50 cm) in the Shallows. Even by 1960 there were a few fish in survey catches exceeding 60 cm long from these areas. Wastage was consequently proportionately lower. The general decrease in sizes since 1960 (Fig. 9) is also apparent, and has the effect of increasing the proportion of wastage.

Marked instances of size or age segregation were noted in May and June in particular areas. In May and June, just after spawning, large fish tended to remain feeding on shallow, sandy banks. The best documented area was just northwest of the Magdalen Islands on shallow grounds. Tows in early June 1960 caught large plaice in 10–20 fath, whereas off the banks in the same general area the fish were of normal size frequency (Fig. 16). Concentrations of this type large enough to support commercial trips no longer occur.

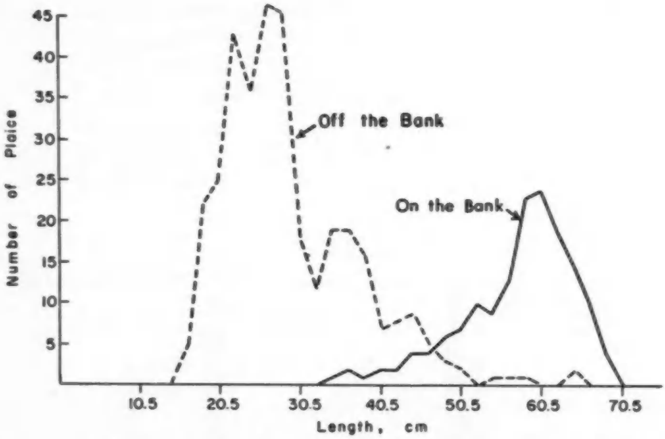

FIG. 16. Size frequencies of American plaice in June 1960 in the Magdalen Island Banks area illustrating seasonal segregation of old, post-spawning fish. *Broken line*, 30–40 fath (50–70 m); *solid line*, 10–20 fath (18–35 m).

Large plaice such as these are usually "jellied" (Templeman and Andrews, 1956), and would be discarded, but since so few large plaice are now present, the proportion wasted as "jellied" is insignificant.

WASTAGE ESTIMATES FROM RESEARCH DATA

Survey hauls were used to obtain discard estimates representative of the whole 4T area, for vessels fishing mainly cod as well as for those fishing mainly plaice. The length frequencies of plaice caught with small mesh on all survey stations were combined and averaged in numbers per half-hour haul (dotted line, Fig. 17). Knowing the retention properties of 4.5- and 5.5-inch meshes, the theoretical catch which would be made by each of these nets was calculated. From known discard practices of commercial vessels, expected numbers and sizes landed were estimated. The percentage of fish discarded for each type of codend was then computed. However, if the trips made by commercial vessels were plaice-oriented, computed (theoretical) landings by commercial vessels would be too small. For cod-oriented trips, however, the plaice discards estimates obtained by theoretical survey methods are probably close approximations.

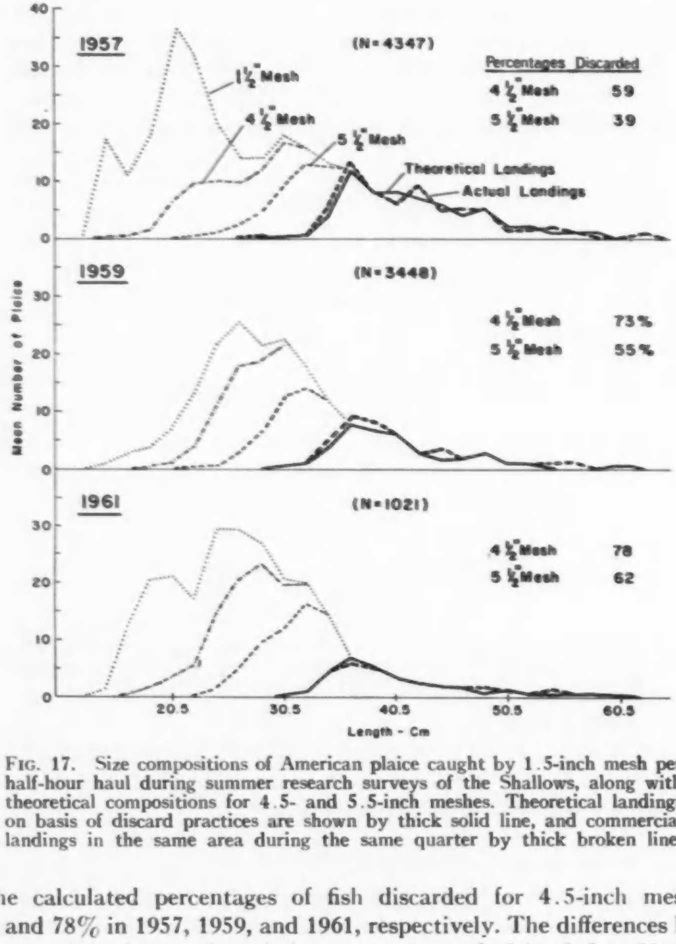

FIG. 17. Size compositions of American plaice caught by 1.5-inch mesh per half-hour haul during summer research surveys of the Shallows, along with theoretical compositions for 4.5- and 5.5-inch meshes. Theoretical landings on basis of discard practices are shown by thick solid line, and commercial landings in the same area during the same quarter by thick broken line.

The calculated percentages of fish discarded for 4.5-inch mesh were 59, 73, and 78% in 1957, 1959, and 1961, respectively. The differences between years are mainly due to the relative proportions of plaice below 30 cm long. Considerable wastage could be prevented by use of a 5.5-inch mesh. The theoretical figures obtained for the years 1959 and 1961 fall within the range of values obtained from observations taken on commercial vessels (Fig. 14).

Survival of Discarded Plaice

What is the actual fate of small plaice discarded at sea from commercial vessels? During commercial operations the sequence of events (mending the net, sorting, gutting of roundfish, etc.) generally results in plaice being exposed on deck for considerable periods of time. Repeated observations showed that the average minimum time small plaice lie on deck prior to dumping is 45 min.

The probability of young plaice surviving after muscular fatigue during capture and exposure on deck was tested in October 1960. Recently caught plaice from 10 to 29 cm long were left out of water on deck for varying amounts of time. The length group 10–29 cm is representative of the category "discards," whereas most fish over 30 cm long would be kept. At intervals of from 5 to 45 min, fish were then placed in tanks of running sea water and observed for at least 50 min. The numbers of plaice surviving and dead at the end of the observation period were then recorded.

These experiments showed that survival time was positively related to size (Fig. 18). At the end of 30 min exposure on deck, 100% of the smaller sizes (10–19 cm) and 95% of the intermediate sizes (20–29 cm) had died. All plaice were dead at the end of 45 min exposure. Although some seasonal differences might be expected, this indicates that almost all plaice less than 30 cm long are dead when returned to sea from commercial boats.

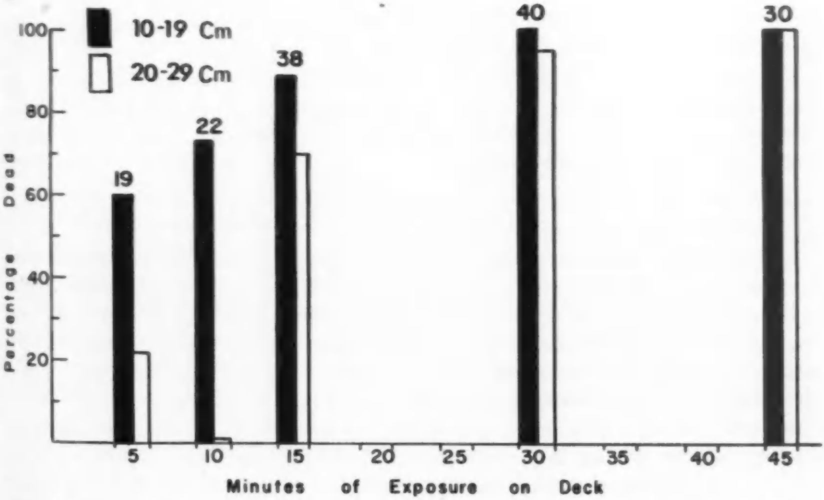

FIG. 18. The survival of two size-groups of American plaice in relation to time of exposure out of water after ½-hr otter-trawl tows. The number of fish in each sample is shown above the bar.

NATURAL FACTORS INFLUENCING YIELD

In assessing the status of a stock it is generally difficult to elucidate the roles of environmental factors (other than the fishery) which may have been active during the period of exploitation. However, for the Magdalen Shallows, information from various sources suggest that sweeping environmental changes have not occurred.

DISTRIBUTION

Cod and plaice have been the two most abundant species of groundfish since at least 1915. Hjort (1919) writes for example, "we encountered a characteristic occurrence of eggs of gadoid species, in particular of the cod proper and of the flatfish *Drepanopsetta* (*Hippoglossoides*) all over the banks in what we have called Madgalen Bay. Besides these greatly predominating species, we found...." Dannevig (1919) in his June plankton tows, found plaice eggs in places and numbers similar to those obtained from current plankton studies (Kennedy and Powles, MS, 1964). Hjort (1919) further points out the names of various other species of fish taken in the Shallows. On the whole, these species are the same ones taken currently.

Distribution of adult plaice in the Shallows has changed little since 1915 (compare Huntsman, 1918 and Powles, 1965). Dannegig (1919) hypothesized the nature of their seasonal movements, a conjecture which has since been corroborated by Powles (1965). It seems likely that species composition and distribution of eggs and larvae have also remained relatively unchanged. Furthermore, the general hydrographic conditions described by Bjerkan (1919) showed stratifications, temperatures, and salinity ranges similar to those currently encountered in the Shallows. It is true that some cooling may have taken place since these earlier years, but Lauzier and Marcotte (1965) have been unable to demonstrate any surface temperature fluctuations of major significance during 1947–59. It is possible that bottom temperatures could change, but plaice has a wide temperature tolerance, and should withstand any long-term or slow changes.

GROWTH AND SURVIVAL

During the 1950's and early 1960's extreme fluctuations in growth rate were not apparent, although growth rate was slightly more rapid than in the time of Huntsman's (1918) studies. Natural factors affecting growth rate and abundance, not already discussed, are: type and availability of food, diseases, competition, density, and predation. Diet overlap occurs between small cod and small plaice, but between adult plaice and cod it is slight (Powles, 1965). The diets of adult and young plaice differ. No extreme fluctuations in numbers of young plaice during a 10-year period (1957–66) has been demonstratable (Powles, unpublished data), only a reduction in mean size. Since molluscs and echinoderms (the food of mature or large plaice) are not utilized by small fish, there is no reason to suspect an increased food resource, or less intraspecific

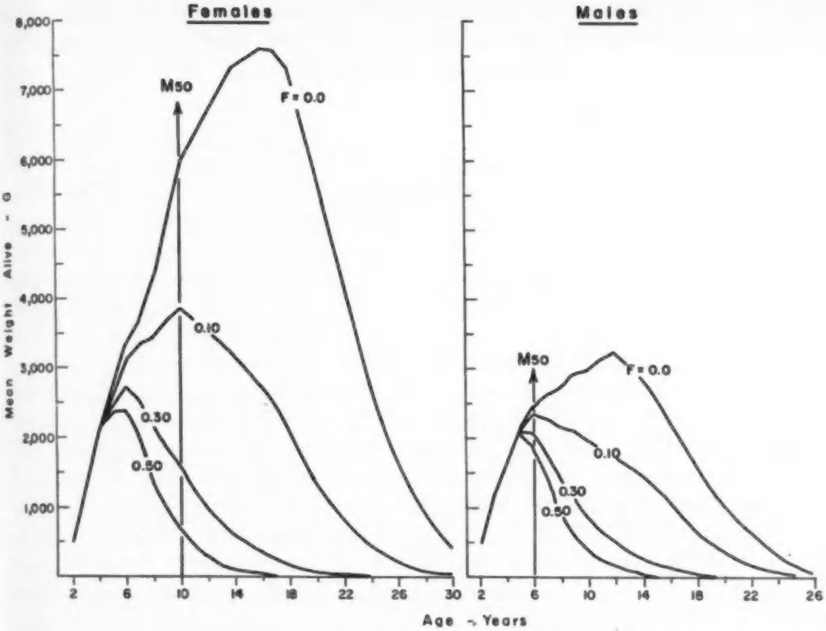

FIG. 19. Weights of female and male plaice remaining at each age for four different rates of fishing (F) assuming constant recruitment of 500 g at age 2. M50 is the 50% maturity age.

competition. Plaice diseases and epidemics within the Shallows have not occurred. We are left, therefore, with predation by cod and effects of the commercial fishery as the two most important known factors.

YIELD PER RECRUIT MODELS

METHODS

On the basis of "best estimates" of parameters, models were first constructed to demonstrate separately the effects of fishing, age at entry to the fishery (mesh size), and cod predation on the Shallows plaice stock. The final models incorporate such factors and show optimum yields for various rates of fishing and age of entry. Ricker models (Ricker, 1958, p. 210) illustrate the relative importance of wastage and predation on the stock. Concerning recruitment stability, there have been minor fluctuations in stock density, judging by the history of the fishery, but no really large year-classes have been observed since those of 1950 and 1943.

Some modifications were made to improve accuracy in the computations. The growth rate of plaice differs with sex (Powles, 1965). For this reason mesh selection ogives also differ with sex. Yields for each sex were therefore computed separately (since natural mortality also differs with sex) and then combined.

The first approximation models (Fig. 20) illustrate in one figure the concomitant effects of altering mesh size and age at entry, the results for the two sexes being combined.

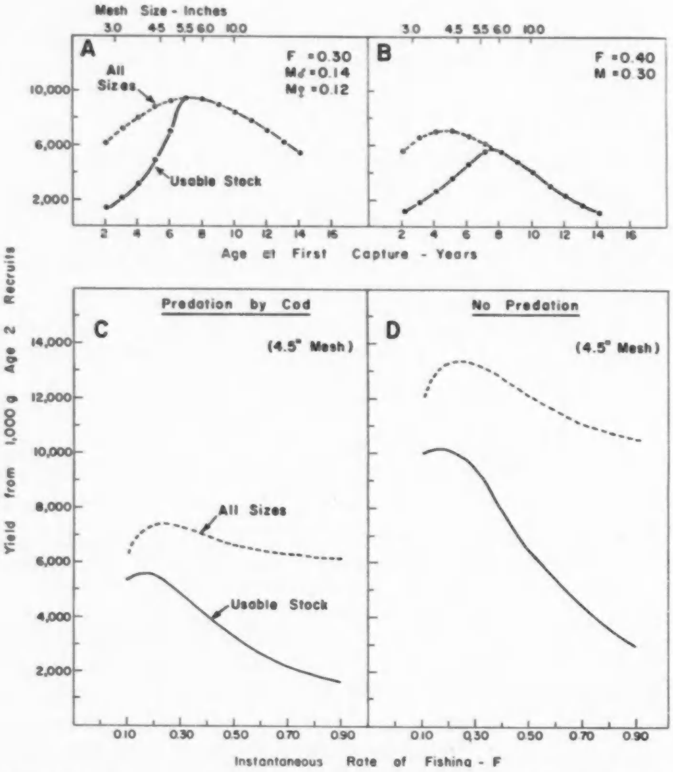

FIG. 20. First approximations of total and usable yields of American plaice with: A, B, different age at entry into the fishery, natural mortality, and rate of fishing; C, mesh size constant, natural mortality varied with age to include cod predation; D, mesh size constant, no predation, natural mortality constant at M = 0.14.

The arbitrary recruitment at age 2 was held constant at 500 g of males and 500 g of females. The year was divided into halves, with commercial fishing occurring only during May–November. During this half of the year, estimated predation rates were included in the natural mortality rates of small plaice under 30 cm long. Lengths were converted to weights from the length-weight relationship (Powles, 1967) and instantaneous growth rate was expressed as the difference between the natural logarithms of these weights.

Only those plaice above the 100% retention age were subjected to the full rate of fishing in the models. The fishing rates for smaller plaice were computed separately from mesh-selection curves, and were dependent on age. It was further assumed that no plaice discarded at sea survived, since this was the result of the survival experiments carried out.

Effects of Fishing

Ricker (1963) points out that a fish population which before exploitation includes upwards of 12–15 age-groups in appreciable quantities (slow growth and low natural mortality) is very sensitive even to fairly low rates of fishing. Figure 19 illustrates that American plaice follow this pattern. This model indicates that a fishing rate of 10% per annum theoretically halves the structure of the standing stock of females. The effect is not as great on males, but reduces their stock by about a third.

Of note also is the eventual result of a moderate fishing rate on the spawning stock. Fishing at a rate of 26% per annum ($F = 0.30$) would eventually reduce the spawning stock of females to about 10% of its original weight. The structure of the current stock would indicate that such a state has not yet been reached. The fishery has operated for over 15 years, however, which is enough time for more than half the year-classes to have been subjected to progressively increasing rates of fishing. Since this study suggests that current instantaneous rates of fishing are about $F = 0.46$, appraisals of the stock, including spawners, should be continued.

Yield and Mesh Size

The effects of fishing must be considered in conjunction with mesh size. To examine effects of age at entry to the fishery (mesh size) yield was computed from 1000 g of males and females, combined in the sex ratio observed for 2-year-olds during research surveys (55:45, males predominating). This model is a first approximation, since a combined growth rate for males and females was used. By this means, special selection properties for each sex were unnecessary. Furthermore, it provides a valuable check on the final model, in which yield for each sex was computed separately, starting with 500 g of males and 500 g of females. Actually, it was the only way in which yield by mesh size and age at entry could be shown together in one model because whereas size selection does not vary with sex, age selection (Fig. 4) does. Figure 20A illustrates effects on yield of mesh size and age at entry. In the current fishery, only larger fish are marketed, so usable yield is shown separately. Optimum yields of usable stock result with ages at entry of 7 and 8 years (5.5-inch mesh). Higher fishing and natural mortality rates alter usable and total yield (Fig. 20B), and raise the optimum age at entry to 8 years.

Effects of Predation

Figures 20C and D illustrate differences in yield between two stocks: one in which cod predation rates were incorporated into the natural mortality rate of young fish and one in which predation was omitted. Yields in models without predation were almost double. The grazing rate (=predation) of cod on plaice used was the instantaneous rate of 0.16, obtained in 1961 (Powles, 1965, p. 578).

OPTIMUM YIELDS

The preceding models illustrated effects of two important factors on Shallows plaice yield: (1) fishing rate and mesh size; and (2) cod predation. Finally, more detailed models which combine best estimates of predation rate, fishing rate (wastage), and age at entry to the fishery were computed (Fig. 21).

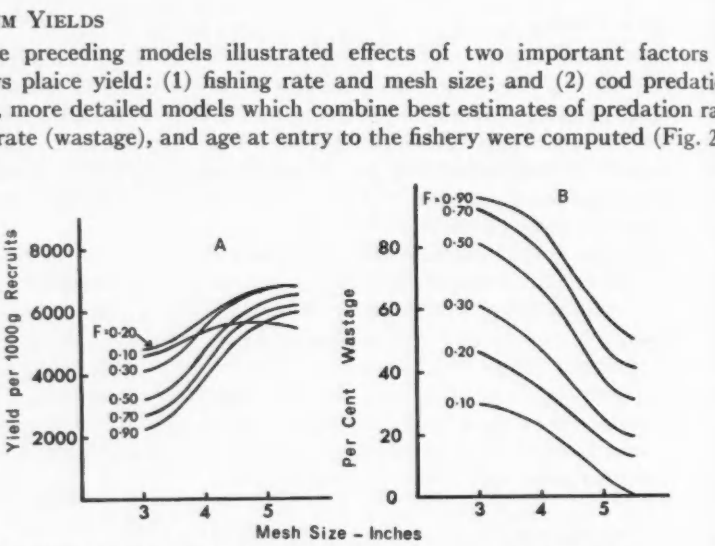

FIG. 21. (A) Total yields of American plaice by mesh size and instantaneous rates of fishing (F); (B) proportion of discards by mesh size and fishing rate.

In considering sustainable yield of this plaice fishery and discounting possible changes in recruitment and growth, the most complicated factors are the disparities between usable and total yields, which vary with both mesh size and rate of fishing. Optimum sustainable yield would be achieved with 5 or 5.5-inch mesh and rate of fishing at F = 0.20 or 0.30 (Fig. 21A). Under these conditions the wastage would be in the range of 20 to 40% by weight (Fig. 21B). No increase in usable yield could be expected by the use of smaller meshes, say below 4.5 inches. Higher rates of fishing would result in increasing disparity between total and usable yields, even with large mesh sizes. At F = 0.50 and mesh size of 5.5 inches, for example, wastage would be 30% by weight. Unless cull practices change, intensified fishing will result in increased wastage.

An increase in rate of fishing from F = 0.3 to F = 0.5 results in a 10% decrease in total yield for 4.5-inch mesh. Increasing fishing to F = 0.7 would result in a decrease of about 16% of the yield expected at F = 0.3. Ideally the stock should therefore be fished with a 4.5-inch mesh at an instantaneous rate of F = 0.3, where yields would remain close to optimum. However, the current rate of fishing probably exceeds F = 0.3. From a long-term point of view, we cannot expect increased landings unless smaller fish are marketed.

DISCUSSION AND CONCLUSIONS

Previous studies (Powles, 1965) have indicated that one of the factors affecting catches of American plaice in the Magdalen Shallows is the seasonal distribution of the stock. It should be reiterated that, since the largest concentrations of plaice in summer overlap cod fishing grounds (Fig. 2), no lessening of fishing effort can be expected in the future.

During winter and early spring, plaice could be especially vulnerable to fishing because they are more concentrated than in summer. However, winter fishing conditions are usually poor because of high winds and ice. The models in this study take no account of future seasonal changes in fishing practices. Should these change radically, new assessments would have to be made.

The tagging results for plaice gave estimates of parameters which appear reliable. The estimates of natural mortality (instantaneous rate from 0.09 to 0.27) compared well with the estimate made from the virgin stock for ages fully exposed to the fishery. Similarly, the instantaneous rate of fishing (0.46) compared well with the estimate obtained by subtracting the natural mortality from total mortality estimates of research-vessel catch curves. The rate of fishing prior to 1957 is not known accurately, but was less than 0.36, the estimate computed for the 1957–62 period. During 1962–66 the rate of fishing increased to $F = 0.46$. The rate of fishing for cod in the Shallows for similar dates is well in excess of the estimate obtained for plaice (L. M. Dickie, personal communication) but cod are exposed to heavy fishing during spring and winter months in deep water outside the Shallows, whereas plaice are not.

Judging from models, sustained yields of plaice cannot be expected to increase, even with increased rates of fishing. One factor limiting the annual yields is the heavy wastage of young plaice at sea. Raising the mesh size from 4.5 to 5.5 inches would result in the loss of large quantities of cod. The 50% selection length of cod for 5.5-inch mesh is 49 cm. Since modes in length frequency of Shallows cod (1963–65) are around 49 cm, catches of cod would be reduced immediately by about 50% in weight. A loss to fishermen of some 30 million lb would be the result of a change to 5.5 -inch mesh. Even a long-term increase of, say, 30% (i.e., 7–10 million lb) in plaice landings could not possibly offset such cod losses, so it is completely impractical to think in terms of a 5.5-inch mesh. However, plaice landings with 4.5-inch mesh could be increased by marketing smaller fish, down to age 6, since 90% of those from age 6 to 8 are currently discarded. Little use is being made of fish as small as these on the east coast. The price for fishmeal is too unattractive, there are too few buyers for animal food, and small fish are not marketed whole as they are in some European countries. Until this situation changes, therefore, it is unlikely that large amounts of small fish will be landed.

Of the few possible restrictive measures available to conserve this slow-growing species, none can be practically favoured. Generally, drastic restrictions are employed only when it can be shown that they will increase the catch to keep it at a safe level, but in the present instance it is impractical and probably unnecessary to increase mesh size. Spawning occurs before the fishing season opens, so that no restrictive measures are needed for closure of the spawning season. There are apparently no particular nursery grounds for small plaice, so that closures of such areas are impossible. A voluntary method of conservation would be to release immediately all fish below commercial size when they are brought on deck. However, this would pose problems for fishermen, who would probably consider the scheme impractical or unnecessary.

In many parts of the Shallows plaice are not sufficiently numerous for profitable fishing. They may also be scattered over rough bottom where small line fisheries for cod are carried out, and in both of these circumstances the fish are not really vulnerable to fishing rates computed for the main plaice grounds. Since the area is large in relation to the number of vessels fishing in it, and since spawning takes place over a wide area, the spawning stock is not threatened but could be reduced at present levels of fishing. If fishing effort is further expanded, mean sizes will continue to decrease. Female plaice do not mature until about age 8 (50% are not mature until age 10) so that it is conceivable that the spawning stock may eventually be further reduced. There are still insufficient data, however, to assess the effects of a decrease in the plaice spawning stock. Interrelationships between fecundity, egg and larval mortality, and competition should be the topics of future studies, as should continued observations on the growth, size at maturity, and conditions affecting recruitment. Major changes in feeding habits and food resources could have far-reaching effects as well. If, for example, as suggested by Paloheimo and Kohler (1968) the absolute productivity of small cod has increased in the Magdalen Shallows, several effects could be felt by the plaice stock. The most likely effect is that since small cod and plaice compete for the same food (Powles, 1965), then increased competition would favour cod because of their larger numbers. Such an interaction would tend to reinforce the dominance of cod over plaice in terms of food competition. Study of the trophic interrelationships between the two species would elucidate these points.

The reasons for the productivity and success of the cod over the years in comparison with the American plaice have not been studied as yet. One of the important factors is that cod is an intermittent spawner, whereas plaice has a short spawning period in early spring. This offers inherent adaptive advantages to cod. However, the larval ecology of the two species has not been well documented, and taxonomic, behavioural, and physiological aspects of the seasonal interrelationships between the two species requires attention before definitive conclusions can be made concerning sustainable yield.

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REFERENCES

Beverton, R. J. H., and S. J. Holt. 1957. On the dynamics of exploited fish populations. U.K. Min. Agr. Fish., Fish. Invest. (Ser. 2) 19: 533 p.

Bjerkan, P. 1919. Results of the hydrographical observations made by Dr. Johan Hjort in the Canadian Atlantic waters during the year 1915. Canadian Fisheries Expedition 1914–1915. Canada, Dept. Naval Service, Ottawa.

Dannevig, A. 1919. Canadian fish-eggs and larvae. Canadian Fisheries Expedition 1914–1915. Canada, Dept. Naval Service, Ottawa.

Hjort, J. 1919. Introduction to the Canadian Fisheries Expedition, 1914–1915. Canada, Dept. Naval Service, Ottawa.

Huntsman, A. G. 1918. Histories of new food fishes. I. The Canadian plaice. Bull. Biol. Bd. Canada 1. 32 p.

International Commission for the North Atlantic Fisheries. 1954–67. Statistical bulletin.

Jean, Y. 1963. Discards of fish at sea by northern New Brunswick draggers. J. Fish. Res. Bd. Canada 20: 497–524.

Kennedy, V. S., and P. M. Powles. MS, 1964. Plankton collections from the western Gulf of St. Lawrence and central Nova Scotian banks, 1958 to 1962. Fish. Res. Bd. Canada, MS Rept. (Biol.) 799. 57 p.

Lauzier, L. M., and A. Marcotte. 1965. Comparison du climat marin de Grande-Rivière (Baie des Chaleurs) avec celui d'autres stations de la côte atlantique. J. Fish. Res. Bd. Canada 22: 1321–1334.

Margetts, A. R. 1949. Experimental comparison of fishing capacities of Danish-seiners and trawlers. Rappt. Proces-Verbaux-Conseil Perm. Intern. Exploration Mer 125: 82 p.

Mousette, M., F. D. McCracken, and A. Marcotte. 1965. Distribution of cod catches by commercial vessels in the Gulf of St. Lawrence 1960–1962. Fish. Res. Bd. Canada, St. Andrews Gen. Ser. Circ. 44. 16 p.

Paloheimo, J. E. 1958. Determination of natural and fishing mortalities of cod and haddock from analysis of tag records off western Nova Scotia. J. Fish. Res. Bd. Canada 15: 1371–1381.

Paloheimo, J. E., and A. C. Kohler. 1968. Analysis of the southern Gulf of St. Lawrence cod population. J. Fish. Res. Bd. Canada 25: 555–578.

Powles, P. M. MS, 1961. Catch comparisons between Danish seiner and dragger. Fish. Res. Bd. Canada, Biol. Sta. St. Andrews, N.B., Ann. Rept. and Investigators' Summaries, 1961–62. 227 p.

MS, 1964. Some factors affecting stocks and landings of American plaice (*Hippoglossoides platessoides* F.) in the southwestern Gulf of St. Lawrence. Ph.D. Thesis. McGill University, Montreal, Que.

1965. Life history and ecology of American plaice (*Hippoglossoides platessoides* F.) in the Magdalen Shallows. J. Fish. Res. Bd. Canada 22: 565–598.

1967. Length-weight relationships for *Hippoglossoides*, *Glyptocephalus* and *Limanda* in Division 4. Intern. Comm. Northwest Atlantic Fish. Res. Bull. 4: 121–123.

Ricker, W. E. 1958. Handbook of computations for biological statistics of fish population dynamics. J. Fish. Res. Bd. Canada 20: 257–264.

Robson, D. S., and D. G. Chapman. 1961. Catch curves and mortality rates. Trans. Am. Fish. Soc. 90: 181–189.

Templeman, W., and G. L. Andrews. 1956. Jellied condition in the American plaice, *Hippoglossoides platessoides* F. J. Fish. Res. Bd. Canada 13: 147–182.

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A New Species of *Acantholiparis* (Pisces: Liparidae) from the Eastern Subarctic Pacific Region, with Distribution Notes for the Genus

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GRINOLS, RICHARD B. 1969. A new species of *Acantholiparis* (Pisces: Liparidae) from the eastern subarctic Pacific region, with distribution notes for the genus. J. Fish. Res. Bd. Canada 26: 1237–1242.

Acantholiparis caecus is described for the first time and reported to occur off the northern Oregon coast. The range of its congener, *A. opercularis*, is extended south off Newport, Oregon.

Received September 16, 1968

INTRODUCTION

IN A JOINT PROGRAM of the U.S. Bureau of Commercial Fisheries and the Atomic Energy Commission, about 2000 specimens of fishes have been collected in bottom trawling gear within or seaward from the Columbia River plume in the eastern subarctic Pacific region (defined by Dodimead et al., 1963). Among these specimens are two examples of a new liparid species referable to the viviparous fish genus *Acantholiparis* revised from Burke (1930). Two additional specimens of the new species were kindly made available from the oceanographic collections at Oregon State University by Dr William Pearcy and Rodney Eagle.

Genus *Acantholiparis* Gilbert and Burke 1912

Disc absent; single tubular nostril; teeth simple, in banded pattern; suprabranchial, pre- and post-nasal pores single; opercular arms projecting as strong spines supporting opercular flap in conjunction with "free" posterior arm of suboperculum; branchiostegals 6 (2 + 4).

Acantholiparis caecus sp.nov.

(Fig. 1)

Holotype — Male 58.6 mm in standard length. AEC Station No. 32, 70-ft shrimp trawl, 2122 m, 45°39′N, 125°18′W, June 17, 1966, deposited at U.S. National Museum, Washington, D.C. (Cat. No. US 202175).

Paratypes — Female 57.0 mm in standard length. AEC Station No. 26, 70-ft shrimp trawl, 1554 m, 45°54′N, 125°05′W, May 15, 1963; deposited at University of Washington College

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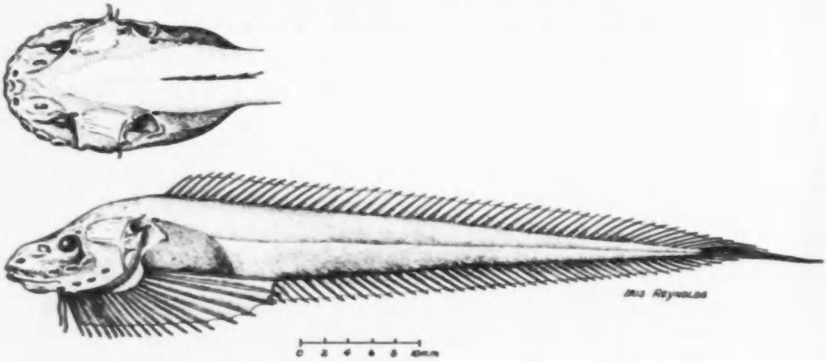

FIG. 1. *Acantholiparis caecus* holotype, showing arrangement of cephalic sensory pores.

of Fisheries, Seattle, Wash. (Cat. No. UW 17042). Female 46.0 mm in standard length, and male 48.0 mm in standard length, both taken from a haul made in 1573 m, 45°50′N, 125°14′W, July 26, 1967, deposited at Oregon State University, Dept. of Oceanography, Corvallis, Ore. (Cat. No. OSU OTB 133).

Diagnosis — The new species differs from its congener, *A. opercularis*, by having well-defined pyloric caeca (Fig. 2); fewer premaxillary teeth beginning anteriorly with a mesiad patch of teeth variously enlarged to greater than twice the size of those contained within the spatially separate and shortened posterior band (Fig. 3, Table 1); more median meristic elements.

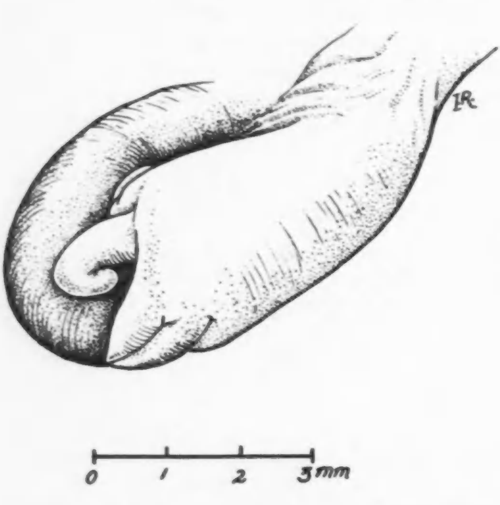

FIG. 2. Pyloric region of alimentary tract in *A. caecus* holotype.

The soft bodies of liparid fishes nearly preclude conclusive morphometric diagnosis; however, the new species appears to have a longer head and wider gill opening, more upper jaw overhang and a greater dorsal overlap on the caudal than its congener (Table 1).

Methods — Counts and measurements were made from the left side of the specimen wherever possible, the latter being recorded to the nearest 0.1 mm (by standards outlined in Hubbs

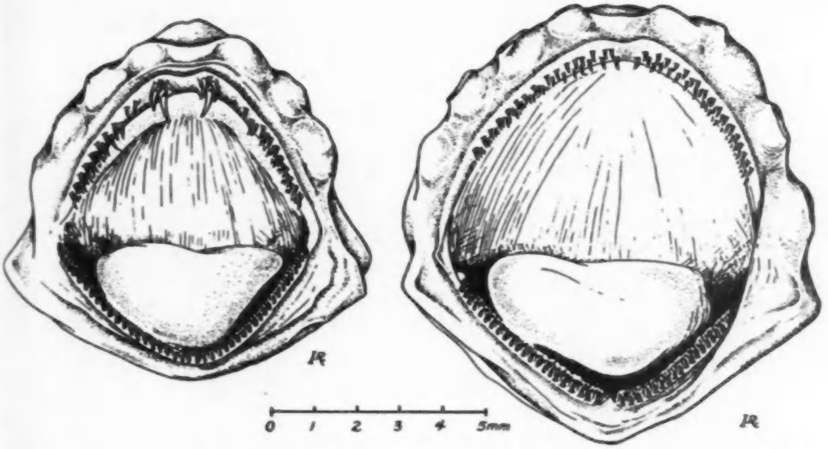

FIG. 3. Arrangement of premaxillary teeth (left) *A. caecus* holotype, and (right) *A. opercularis* (OSU OT 155).

and Lagler, 1958). Total vertebrae count included complete centra plus the urostyle. Complete data are recorded in Table 1.

Description — Body elongate, tapering, and covered with loose, unsquamated epithelium; maximum depth obtained midpectoral. Head and trunk region ventrally depressed. Gape extending posteriorly to a point below posterior margin of pupil. Premaxillary with two contrasting denticle patterns. Anterior mesiad denticle patch containing 4–10 enlarged, recurved conical teeth, greater than twice the size of those within posterior band. Latter with uniformly sized fine conical teeth, recurved distally; 8–11 rows in each band; 1–7 teeth in each row (Fig. 3). Mandible covered by enlarged band of uniformly sized, fine, recurved conical teeth, comparable in size and shape to those in premaxillary band; 15–23 rows in each band; 1–7 teeth in each row; occasional tooth displaced from pattern. Vomer and palatines edentate. Nostril tubular, darkly pigmented, and closer to eye than to tip of snout. Opercle entire, possessing four spines that lend support to opercular flap (Fig. 4). Preopercle sculptured on ventral arm with three large, cavernous pores. Subopercle anteriorly originating loosely in skin between preopercle and ceratohyal (at about origin of the third branchiostegal ray); terminating posteriorly at preopercle-opercle articulation. Branchiostegals and more dorsally situated suboperculum folding together to collectively provide primary support for branchiostegal membrane; pseudobranchiae and thymus absent. Branchial aperture entirely above pectoral fin. Gill rakers discernible on lower arm of gill arch as rudimentary blunt knobs with distal spinous projections; no slit behind fourth arch. Dorsal origin above midpoint of pectoral fin (measured from lower insertion); anal fin beginning beneath 8th dorsal element. Caudal fin elongate; last dorsal ray overlying less than one-half length of fin. Pectoral fin unnotched; rays progressively shortened anteriorly; two ventralmost elements entire, free from fin membrane; intermediate elements more widely spaced than those on either side. Disc absent. Anus positioned beneath dorsal origin about midpoint of pectoral, anterior to origin of anal. Testes bilobed, positioned on either side of urinary duct, occupying posterior one-third of coelomic cavity. Four to five pyloric caeca developed (Fig. 2). Head and body sparsely pigmented except for black on lips, fin emargination, and abdominal region; mouth, branchial chamber, peritoneum, intestine, and stomach all dusky. Live external coloration on head and body dusky red.

Etymology — The trivial name *caecus* is made in reference to the distinctive pyloric caeca possessed by the new species.

TABLE 1. Morphological comparisons between two eastern subarctic Pacific species of *Acantholiparis*. Diagnosis based upon characters in italics.

Character	*A. opercularis*[a]	*A. caecus*[b]
Dorsal rays } Not including caudal	45–46	48–52
Anal rays }	38–40	43–45
Caudal rays	8–9	8
Pectoral rays	21–24	21
Total vertebrae	50	53–54
Caudal vertebrae	41	45–46
Gill rakers	8–10	10–11
Gill arches	4	4
Branchiostegals	2+4	2–3+4
Cephalic pores:		
Prenasal (supraorbital)	1	1
Infraorbital	6	6
Postorbital	1	1
Preoperculo-mandibular	5–6	6
Suprabranchial	1	1
Pyloric caeca	0	4–6
Dentition (rows in band)		
Premaxillary	ca.16–21	ca.10–12
Mandible	ca.22–25	ca.18–23
Total length (*mm*)	73.5–83.4	56.0–70.0
Standard length (*mm*)	62.4–69.1	46.0–58.6
Head length (to posterior edge of gill opening)[c]	21.7–21.8	22.7–24.0
Head width	22.4–22.9	17.1–22.8
Max head depth	10.1–10.9	10.9–12.1
Length of gill opening	4.2	5.1–5.8
Length of upper jaw	10.3–11.9	11.5–12.3
Length of lower jaw	9.3–10.4	10.2–11.2
Length of jaw overhang	1.4–1.9	2.0–2.6
Length of snout	7.1–7.9	7.0–8.0
Interorbital width	3.8–4.2	4.0–4.6
Orbital width	4.6–5.3	4.8–5.6
Width nostril (horizontal)	1.1–1.5	1.0–2.2
Max body depth (taken at midpectoral length)	8.7–11.2	10.8–14.0
Length dorsal base	75.3–78.3	76.3–79.5
Dorsal overlap on caudal	7.2–8.7	8.8–9.8
Length caudal	17.6–20.3	18.8–21.7
Length anal base	65.7–70.6	64.0–71.3
Anal overlap on caudal	4.3–4.8	3.3–5.2
Length pectoral		
Upper insertion and rays	17.4–20.7	14.6–21.5
Lower insertion and rays	4.3–8.7	1.1–6.0
Width pectoral base	14.0–14.4	13.8–15.7
Predorsal length	23.7–24.8	24.6–29.6
Preanal length	32.9–34.2	31.9–36.5
Snout to pectoral		
To upper insertion	20.4–22.3	20.1–22.8
To lower insertion	8.0–8.3	7.3–9.1
Snout to center of anus[d]	20.4–22.3	18.1–25.2
Anus-anal interspace[d]	11.7–13.5	10.9–15.4

[a] Two specimens: 69 mm standard length female emended from Grinols (1966); 62 mm standard length male from Oregon State University (OSU OT 155)

[b] Taken from four type specimens.

[c] These and later measurements are given as percentages of standard lengths.

[d] Coelomic cavity opened; therefore, position of anus not consistent or reliable.

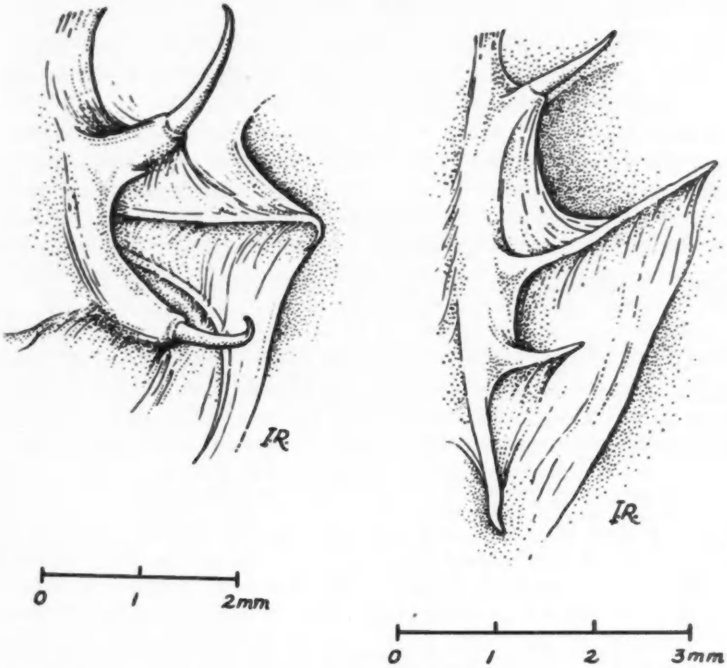

FIG. 4. Opercular spines on (left) *A. opercularis* (OSU OT 155) and (right) *A. caecus* holotype.

DISTRIBUTION NOTES FOR THE GENUS

Acantholiparis caecus is known from four specimens taken seaward from Tillamook Rock, Oregon, at 1554–2122 m. Because no opening–closing device was used in our surveys the exact depth of capture is always in question. However, owing to the relative inefficiency of the gear during shooting and retrieval, it is likely the specimens were caught at depth. Furthermore the "projecting shovel-shaped snout" (Burke, 1930), stubbled gill rakers, opercular spinulation, and well-developed denticles suggest that the new species is adapted to a benthonic rather than a pelagic environment.

Acantholiparis opercularis, previously reported on the basis of four specimens, has been reported to range from off the southeastern tip of Kamchatka (52°38′N, 158°50′E) in 1247 m westward to below the Gulf of Alaska, near the Shumagin Islands (53°58′N, 159°31′W), in 3609 m and southward to off the northern Oregon coast (45°40′N, 124°55′W) in 1372 m (Grinols, 1966). A fifth specimen, a previously unreported female (62.4 mm standard length) (Table 1, Fig. 3 and 4) taken January 10, 1967, by Oregon State University, emends the southward distribution to 65 miles seaward from Newport, Oregon (44°37′N, 125°35′W) in 2800 m.

VARIATION

All the acantholiparid species studied appear to be mature adults; half of the specimens are within 3 mm in standard length, hopefully a reflection of similar age. Both sexes are represented in the collections.

Comparison within species — *Acantholiparis caecus* type specimens agree in most characters examined (Table 1). The holotype of *A. caecus* possesses a unique opercular configuration consisting of a main vertical arm radiating four strong retrorse spines. Other acantholiparid specimens examined had only two or three main retrorse spines off the main beam; the remaining support for the branchial membrane disarticulated from and positioned beneath the opercle (Fig. 4).

Variation among four specimens of *A. opercularis* was summarized by Grinols (1966). The recently acquired Oregon State University (OSU) specimen varies in about the same direction and magnitude from the type description of *A. opercularis* (Gilbert and Burke, 1912; Burke, 1930) as did Grinols' (1966) Oregon specimen, except the former (OSU specimen) has larger eyes, longer pectorals, and a greater maximum body depth. The last character (body depth) brings the OSU specimen close to the type description. Additional variation between the two Oregon specimens of this species (Table 1) occurs mainly in the jaw length, in lengths of dorsal and anal bases, in dorsal overlap on caudal, and in pectoral, predorsal, preanal, and prepectoral lengths. It should be emphasized that these characters, with the exception of predorsal length, exhibited greater intra- than inter-specific variation. The significance of this variation cannot be tested on the basis of only a few specimens because most values were obtained from soft, movable parts and lacked consistency. Both the type and Bureau of Commercial Fisheries specimens have more pectoral rays than the Oregon specimen. The latter specimen has a complement of 21 rays, the same number as the new species and the illustration accompanying the Gilbert and Burke (1912) type description.

Comparison between species — In addition to those characters used in the diagnosis for the new species, a review of Table 1 suggests that the new species also has a deeper head, a larger interorbital region, a greater maximum body depth, and a longer predorsal length than *A. opercularis*. However, the range of variation for these characters from the type description of *A. opercularis* given by Gilbert and Burke (1912) and Burke (1930) shows overlap between the two species.

REFERENCES

BURKE, VICTOR. 1930. Revision of the fishes of the family Liparidae. Bull. U.S. Natl. Museum 150. 204 p.

DODIMEAD, A. J., F. FAVORITE, AND T. HIRANO. 1963. Review of oceanography of the subarctic Pacific region. Intern. N. Pacific Fish. Comm., Bull. 13. 195 p.

GILBERT, CHARLES H., AND CHARLES V. BURKE. 1912. Fishes from the Bering Sea and Kamchatka. Bull. [U.S.] Bur. Fish. 30: 31–96.

GRINOLS, RICHARD B. 1966. Southern occurrence of *Acantholiparis opercularis* Gilbert and Burke in the eastern subarctic Pacific region. J. Fish. Res. Bd. Canada 23: 935–937.

HUBBS, CARL L., AND KARL F. LAGLER. 1958. Fishes of the Great Lakes region. Cranbrook Inst. Sci. Bull. 26. 213 p.

Movements and Homing of Cutthroat Trout (*Salmo clarki*) from Open-Water Areas of Yellowstone Lake[1]

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JAHN, LAWRENCE A. 1969. Movements and homing of cutthroat trout (*Salmo clarki*) from open-water areas of Yellowstone Lake. J. Fish. Res. Bd. Canada 26: 1243–1261.

Cutthroat trout (*Salmo clarki*) showed in-season homing after displacement from their spawning tributaries to Yellowstone Lake during June–August 1966 and 1967. Of 300 nonanesthetized trout tagged and displaced from Clear and Cub creeks to three release points (0.5–22.0 km) in the lake and to the mouth of Clear Creek, 38.3% homed, 10.0% strayed, 2.7% were caught by anglers, and the remainder were unaccounted for. Anosmic (olfactory chambers plugged) and blind-anosmic fish homed in significantly lower percentages than nonanesthetized and control groups. Fish released just outside the mouth of the homestream had the shortest average homing time, but the average homing time for fish displaced 22.0 km from the homestream was shorter than for those displaced 5.0 km away. Homing percentages for trout tagged after being tracked individually in the open lake were similar to those for trout in the group tagging experiments, but the former had longer average homing times than the latter.

The directions of orientation of fish tracked in open water were generally toward the homestream, the directions usually coinciding with those of the sun azimuth. However, fish taken from the east side of the lake went west-northwest when tracked late in the afternoon and fish taken from the west side of the lake went east-southeast when tracked in the morning, these directions being away from those of the homestreams. Orientation was affected by current. Mean directions for males and females were generally not significantly different. Average swimming speeds and vector lengths for males and females were about the same. Immature cutthroat trout were trained to use a light source as a reference point for orientation.

Received May 28, 1968

INTRODUCTION

OPEN-WATER MOVEMENTS and in-season homing of mature cutthroat trout (*Salmo clarki*) after displacement from spawning streams of Yellowstone Lake, Wyoming, were studied during the trout spawning seasons (May–August) of 1966 and 1967. The objectives were to compare open-water orientations of cutthroat trout taken from five creeks, and to compare the homing performances of those from two of the creeks. The effect of sun azimuth and

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current on orientation were evaluated. In addition, underwater photographs were made to ascertain if landmarks were visible to the fish. Laboratory experiments were conducted to determine if cutthroat trout could be trained to use light for orientation.

Yellowstone Lake has a surface area of 354 km^2, a maximum depth of 98 m, and a mean depth of 42 m, and is at an altitude of 2358 m (Benson, 1961). It is a good place for homing and movement studies because of its relatively large size and numerous spawning tributaries, and because the cutthroat trout population is not contaminated with salmonids from other sources.

In-season homing of mature cutthroat trout in Yellowstone Lake was observed by McCleave (1967). He displaced trout which were nonanesthetized, blinded, olfactory occluded (anosmic), or used as controls. Homing ability was apparently not affected by blinding, but experiments using anosmic fish were inconclusive. Homing times were longer for blinded fish than for others, indicating the importance of visual cues for homing. An inverse relationship between homing times and the distance fish were displaced was found in one year. In-season homing of cutthroat trout in a Utah reservoir was shown by Platts (1959).

Previous studies on open-water movements of cutthroat trout in Yellowstone Lake showed that orientation was mainly eastward or northward from various release points (Jahn, 1966; McCleave, 1967). This was also true for blind and anosmic trout, which oriented as well as control trout. However, orientation was generally not toward the homestream. For trout tracked after noon the main direction was more southerly than for those tracked in the morning (Jahn, 1966). The sun may have served as a reference point in orientation since trout traveled farther and showed a stronger tendency to go shoreward from a near-shore release point on sunny days than on cloudy days.

METHODS AND MATERIALS

HOMING STUDIES

Four release points (Fig. 1) were used for homing studies. These were located at the mouth of Clear Creek and 0.5 km west-northwest, 5.0 km west, and 22.0 km west-southwest of the mouth. Fish used in homing studies were moving upstream to spawning and were taken from traps on Clear and Cub creeks, located 75 and 150 m, respectively, above their mouths. Travel time to release point 1 was 2–3 min, depending on where experimental fish were obtained and speed of the boat; to point 2, 3–15 min; to point 3, 8–18 min; and to point 4, 30–35 min.

Homing experiments were carried out from June 29 to July 9, 1966, and July 11 to 18, 1967. Groups of 25–30 trout were netted from the trap and with few exceptions were taken to a boat. In a few instances, fish taken from Clear Creek were carried directly to release point 1. Four types of experimental fish were used: anosmic, blind-anosmic, control, and nonanesthetized. Anosmic, blind-anosmic, and control fish were anesthetized in a 40 mg/liter solution of tricaine methanesulfonate (M.S. 222). Fish were olfactory-occluded by blowing a jet of air into the olfactory chambers to free them of foreign material and then petroleum jelly (Vaseline, Chesebrough-Ponds Mfg. Co.) was injected into them with a needleless syringe, until the accessory chamber anterior to the eye was full. An empty syringe was placed against the nares of control fish. Fish were blinded by injecting 0.10–0.15 cc of 3% aqueous benzethonium chloride (Phemerol,

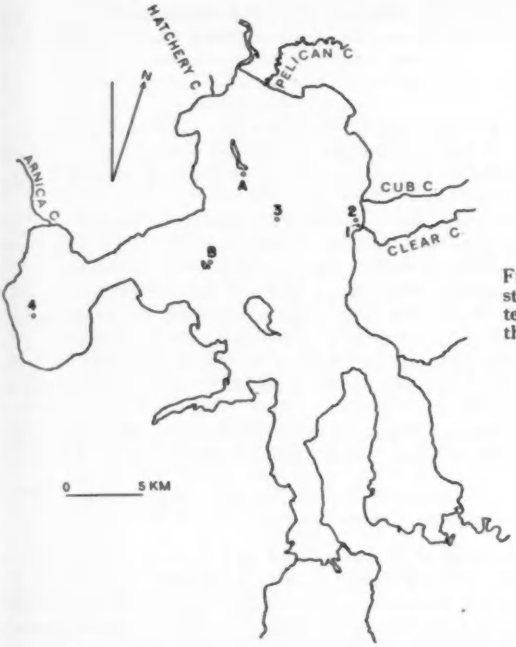

FIG. 1. Yellowstone Lake, showing streams and open-water points (letters A, B) where experimental cutthroat trout were obtained, and the release points (numerals).

Parke, Davis and Co.) into their eyeballs with a syringe (McCleave, 1967). Anesthetized fish were then tagged, placed into a covered stock tank in a boat, taken to the release point, and liberated after recovering from the anesthetic, which was usually by the time the release point was reached.

Nonanesthetized fish were either put into the covered stock tank in the boat, taken to a release point, marked with a numbered alligator clip tag (McCleave et al., 1967), and released, or marked and released at point 1.

Groups of 25 fish were tagged and liberated at each release point.

Most recaptures were obtained from the traps on Clear and Cub creeks. The traps were inspected for tagged fish at various times of the day, but usually in the afternoon. The tag number, date, time of day of removal from the trap, length, and sex of each recaptured fish were recorded. A few tagged fish were taken by anglers. The heads of all recaptured anosmic fish were saved and the olfactory chambers examined to see if the plugs were intact.

FLOAT-TRACKING STUDIES

A float-tracking device consisting of a Styrofoam (Dow Chemical Co.) cube 5 cm on a side connected by 2 m of nylon line to an alligator clip was attached to the dorsal fin of each fish at a release point (Jahn, 1966). Release points 2 and 3 were used for tracking experiments. Fish moving upstream to spawn were obtained from the traps on Clear, Cub, and Pelican (2 km above its mouth) creeks, with a dipnet from Hatchery Creek, and by hook and line from Arnica Creek. Nonspawning fish were caught with hook and line 8.4 km west-northwest (point A) and 10.1 km west-southwest (point B), respectively, of the mouth of Clear Creek (Fig. 1). A few spawned-out fish were also caught at points A and B.

Tracking experiments were conducted from May 31 to July 28, 1966, and June 16 to July 25, 1967. Groups of two to six fish were taken to a release point in a covered tub. Four types of experimental fish were used: nonanesthetized, blind-anosmic, blind, and controls for each type of anesthetized fish. Treatments for each type were the same as for those used in homing studies, except that control fish used in comparison with the blind fish were anesthetized only.

For experiments at point 2, two nonanesthetized fish or one blind and one control fish were liberated 1 min apart, without attempt to orient them. For experiments at point 3, one of three lots was similarly released: two to five nonanesthetized, two blind-anosmic, and three control, or three blind-anosmic and two control fish. After the fish were released a drift drogue was placed in the water to determine currents at the approximate depth the fish were swimming (Jahn, 1966).

Positions of the fish and drift drogue were determined by sighting on landmarks with a sextant. For experiments at point 2, sightings were taken at ¼-hr intervals on each fish and the drogue. In a few instances fish were lost for a time. Experiments were terminated at 2 hr, or when experimental fish reached an area where the bottom of the lake was visible from the surface by the experimenter, or when windy weather prevented accurate sightings. For experiments at point 3, only one sighting was taken on each fish and the drogue — either 1 hr after the experiment began or sooner if the wind interfered with sightings. This terminated an experiment and the fish and drogue were then picked up as quickly as possible. Most fish were recovered and the length and sex of each were determined.

Most spawning fish that were recovered were tagged and released to determine if there were any effects on homing due to float-tracking. Nonspawning fish were cut open to determine sex.

UNDERWATER PHOTOGRAPHS

Underwater photographs were taken at release points 2 and 3 to see if the images of shoreline features could be recorded photographically at the approximate depths (0.5, 1.0, and 2.0 m) at which the fish swam when tracked. Some were also taken from depths of 1.0 and 2.0 m, 20–80 m from shore. A Nikonos All-Weather camera (Ehrenreich Photo-Optical Industries, Inc.) was attached to the end of a pipe 3 m long so the camera faced upward at an angle of 48° from the vertical (toward the edge of the "fish window," Walls, 1942). Light readings for each picture were taken just under the surface of the lake with a Sekonic Model L-86 light meter in a waterproof housing (Ehrenreich Photo-Optical Industries, Inc.). Pictures were taken using black and white Tri-X Pan Film (Eastman Kodak Co.).

TRAINING EXPERIMENTS

An attempt was made to determine if hatchery cutthroat trout (150–213 mm total length) could learn to use a light source in the laboratory as a reference point for orientation. The training tank (2 m in diameter) was similar to that used by Hasler et al. (1958) and contained 16 wedge-shaped boxes facing outward from a central release chamber. The light source was above the edge of the tank at an angle of 50° from the center. The tank was completely enclosed by black curtains which blocked out extraneous light and hid the experimenter from the fish.

All fish were drilled individually at nearly the same time each day. Two fish were subjected to 5 trials per day for 30 consecutive days using a 100-w light bulb and 6 fish 10 trials per day for 10 consecutive days using a 300-w bulb, all fish having every 7th day off. Three fish were drilled to go 90° clockwise from the light source, three 90° counterclockwise, and two toward the light source. Three untrained fish were used as controls.

Each fish was netted from a trough, placed in the central chamber of the tank, and released after it quieted down. During drilling only one of the 16 boxes was made available for the fish to hide in. An electric probe was used to shock and direct the fish to the open box. After the fish entered the box, it was left undisturbed for 1 min and then netted and returned to the central chamber. This process was repeated for the remaining trials. During testing all 16 boxes were available for the fish to hide in, and the numbers of trials were the same as during drilling. The tank and position of the light were rotated after each day's drilling to prevent use of marks on the tank and other cues for orientation. In addition the experimenter changed his position to avoid being used as a reference point. Fish were tested the day after the last drilling session, at the normal drilling time. Those which oriented were retested 30 hr later. Retention of learning was also tested.

ANALYSIS OF DATA

Data from tagging and tracking experiments were analyzed with the aid of a computer. For tagging experiments, chi-square contingency tables (Steel and Torrie, 1960) were used to compare data on homing and straying of each experimental group with those for every other. For tracking experiments, the directions taken by fish were represented as points of equal mass on the circumference of a circle. An empirical mean vector pointing to the center of mass of the distribution of each group of fish was calculated with polar coordinates r (vector length) and a (mean angle) (Batschelet, 1965). Average swimming speed and average length of each fish were calculated for each experimental group. A Rayleigh test (Greenwood and Durand, 1955) was applied to data on each male and female group and to those for the combined groups to determine if the distributions were uniform. A resultant vector F test (Watson and Williams, 1956) was used to compare the combined groups with each other to see if the mean directions were significantly different. An R test (Watson and Williams, 1956; Stephens, 1962) was used to determine if the mean direction of each male and female group and combined group (except nonspawning and spawned-out fish) was toward the homestream. Current direction and sun azimuth were taken as the zero direction for each fish instead of true North. Mean directions, Rayleigh tests, and resultant vector F tests were then recalculated for groups of males and females combined. Summaries of all these tests are given by Batschelet (1965). The normal approximation test (Steel and Torrie, 1960) was used to determine if experimental fish were trained in the laboratory.

RESULTS

HOMING STUDIES

Fish tagged in groups — Of all nonanesthetized fish from Clear Creek 41.0% homed and 7.5% strayed, whereas 33.0% of nonanesthetized fish from Cub Creek homed and 15.0% strayed (Table 1). Of anosmic fish from Clear Creek 12.0% homed and 2.0% strayed whereas 46.0% of control fish homed and 10.0% strayed. Only 4.0% of blind-anosmic fish from Clear Creek homed whereas 40.0% of control fish homed.

Significantly fewer anosmic and blind-anosmic fish from Clear Creek homed and strayed than other experimental groups (Table 2). Significantly more nonanesthetized fish from Clear Creek released at points 3 and 4 homed than nonanesthetized fish from Cub Creek released at point 3.

Average homing times were less for nonanesthetized fish from Clear Creek released at point 1 than for any other group (Table 3). Nonanesthetized fish from Clear Creek homed faster on the average from point 4 (22.0 km) in 1967 than from point 2 or 3 (0.5 and 5.0 km, respectively) in 1966. Nonanesthetized fish from Clear and Cub creeks homed in about the same length of time. The average homing time of anosmic fish from Clear Creek was greater than that of any other experimental group, and 63 hr greater than for control fish. Statistical analyses are not given for homing times since recaptures were not obtained randomly.

Fish tagged after tracking — Homing percentages of fish tagged after tracking were similar to those of counterparts in the group-tagging experiments, except that only 1 of 10 controls for blind-anosmic fish homed after tracking (Table 1).

TABLE 1. Numbers and (in parentheses) percentages of Clear and Cub creek trout treated in various ways that homed, strayed, or were taken by anglers after being tagged in groups or individually after tracking and released at various points, June and July 1966 and 1967.

Year	Release point	Origin creek	Group[a]	No. released	No. recaptured			
					Home	Stray	Angler	Total
				Fish tagged in groups				
1966	2	Clear	NA	50	20(40.0)	3(6.0)	0	23(46.0)
		Cub	NA	50	21(42.0)	7(14.0)	2(4.0)	30(60.0)
	3	Clear	NA	50	24(48.0)	3(6.0)	2(4.0)	29(58.0)
		Cub	NA	50	12(24.0)	8(16.0)	2(4.0)	22(44.0)
1967	1	Clear	NA	50	15(30.0)	5(10.0)	1(2.0)	21(42.0)
	3	Clear	A	50	6(12.0)[b]	1(2.0)	2(4.0)	9(18.0)
			CA	50	23(46.0)	5(10.0)	2(4.0)	30(60.0)
			BA	50	2(4.0)	0	0	2(4.0)
			CBA	25	10(40.0)	0	0	10(40.0)
	4	Clear	NA	50	23(46.0)	4(8.0)	1(2.0)	28(56.0)
				Fish tagged after tracking				
1966	2	Clear	NA	18	6(33.3)	1(5.5)	0	7(38.8)
			B	8	4(50.0)	0	0	4(50.0)
			CB	8	5(62.5)	0	0	5(62.5)
		Cub	NA	14	7(50.0)	0	0	7(50.0)
			B	6	5(83.5)	0	0	5(83.5)
			CB	6	3(50.0)	0	0	3(50.0)
		Pelican	NA	10	0	0	0	0
	3	Clear	NA	14	6(42.8)	0	0	6(42.8)
		Cub	NA	15	3(20.0)	5(33.3)	0	8(53.3)
1967[c]	3	Clear	NA	5	4(80.0)	0	0	4(80.0)
			BA	17	1(5.9)	0	1(5.9)	2(11.8)
			CBA	10	1(10.0)	2(20.0)	1(10.0)	4(40.0)

[a] NA = nonanesthetized; A = anosmic; CA = control anosmic; B = blind; CB = control blind; BA = blind-anosmic; CBA = control blind-anosmic.

[b] Does not include one fish which had one plug missing.

[c] Only those released after the fish traps were permanently installed are considered. Fish from Arnica Creek are not included since there was no trap operated there.

Significantly fewer blind-anosmic fish from Clear Creek homed than all other groups tagged after tracking except nonanesthetized fish from Cub Creek released at point 3 and controls for blind-anosmic fish (Table 4). Blind and control fish from Cub Creek tracked from point 2 homed equally well. No fish from Pelican or Arnica creeks were found among the recaptures; no fish-tight traps were operating in these streams.

The average homing times for blind fish from Clear and Cub creeks were 78 and 146 hr greater, respectively, than for control fish (Table 3). Control and nonanesthetized fish from Clear and Cub creeks homed in about the same time from point 2 in 1966. The average homing times of nonanesthetized

TABLE 2. Homing and straying of fish tagged in groups, showing significantly different comparisons of nonanesthetized fish taken from Cub Creek and of anosmic and blind-anosmic fish from Clear Creek with other groups.

Year	Release point	Origin creek	Group[a]	vs.	Year	Release point	Origin creek	Group[a]	Chi-square[b]
					Homing				
1966	3	Cub	NA		1966	3	Clear	NA	4.72*
					1967	3	"	CA	4.68*
						4	"	NA	3.89*
1967	3	Clear	A		1966	2	"	"	10.42**
							Cub	"	15.22**
						3	Clear	"	17.21**
							Cub	"	3.91*
					1967	1	Clear	"	5.82*
						3	"	CA	17.05**
								CBA	7.00*
						4	"	NA	15.65**
1967	3	Clear	BA		1966	2	"	"	20.53**
							Cub	"	26.59**
						3	Clear	"	28.99**
							Cub	"	11.43**
					1967	1	Clear	"	14.30**
						3	"	CBA	16.07**
								CA	28.80**
						4	"	NA	27.10**
					Straying				
1966	3	Cub	NA		1967	3	Clear	CBA	3.95*
1967	3	Clear	A		1966	2	Cub	NA	7.47*
						3	Cub	"	8.88*
					1967	1	Clear	"	3.92*
						3	"	CA	5.96*
						4	"	NA	3.98*
1967	3	Clear	BA		1966	2	"	"	4.99*
							Cub	"	13.72**
						3	Clear	"	6.26*
							Cub	"	11.78**
					1967	1	Clear	"	7.51*
						3	"	CA	10.30**
						4	"	NA	7.80**

[a] Legend as in Table 1.
[b] *Significant at $P = 0.05$; **significant at $P = 0.01$.

fish from Clear and Cub creeks tagged after tracking in 1966 from point 2 were 20 and 35 hr less, respectively, than those of counterparts tagged after tracking from point 3.

TABLE 3. Times (hr) from release to recapture of Clear and Cub creek trout released during 1966 and 1967.

Year	Release point	Origin creek	Group[a]	No. released	Home time			Stray time		
					No.	Range	Avg	No.	Range	Avg
				Fish tagged in groups						
1966	2	Clear	NA	50	20	8–201	109	3	26–223	94
		Cub	"	50	20[b]	71–226	117	7	8–100	74
	3	Clear	"	50	24	34–392	148	3	32–250	135
		Cub	"	50	12	35–221	131	8	29–249	105
1967	1	Clear	"	50	15	6–142	60	5	19–94	39
	3	Clear	A	50	6	121–219	160	1	113	113
			CA	50	23	22–293	97	5	48–360	165
			BA	50	2[c]	96–120	108	0	0	0
			CBA	25	10	50–312	113	0	0	0
	4	Clear	NA	50	23	45–216	90	4	45–96	61
				Fish tagged after tracking						
1966	2	Clear	NA	18	6	29–462	177	1	297	297
			B	8	4	167–392	263	0	0	0
			CB	8	5	5–339	185	0	0	0
		Cub	NA	14	7	48–340	180	0	0	0
			B	6	5	102–413	302	0	0	0
			CB	6	3	29–340	156	0	0	0
	3	Clear	NA	14	6	104–272	197	0	0	0
		Cub	"	15	3	199–247	215	5	56–103	87
1967	3	Clear	"	5	4	36–159	99	0	0	0
			BA	17	1	339	339	0	0	0
			CBA	10	1	241	241	2	69–214	142

[a] Legend as in Table 1.
[b] Does not include one fish whose tag was found in the bottom of the Cub Creek trap 434 hr after release.
[c] Seen in stream, and not caught in the trap.

TABLE 4. Homing and straying of fish tagged after tracking, showing significantly different comparisons of nonanesthetized fish from Pelican, Clear, and Cub creeks, of blind fish from Cub Creek and of blind-anosmic fish from Clear Creek with other groups. Fish from Arnica Creek were not included since there was no trap operated there.

Year	Release point	Origin creek	Group[a] vs.	Year	Release point	Origin creek	Group[a]	Chi-square[b]
				Homing				
1966	2	Pelican	NA	1966	2	Clear	NA	4.53*
							B	6.42*
							CB	8.65**
						Cub	NA	7.05**
							B	12.12**
							CB	6.15*
					3	Clear	NA	5.71*
				1967	3	"	"	10.90**
		Cub	B	1966	2	"	"	4.10*
					3	Cub	"	4.26*
				1967	3	Clear	CBA	6.19*
1967	3	Clear	BA	1966	2	Clear	NA	4.16*
							B	6.18*
							CB	9.00**
						Cub	NA	7.30**
							B	13.07**
							CB	5.61*
					3	Clear	NA	5.59*
				1967	3	"	NA	11.42**
1967	3	Clear	NA	1967	3	"	CBA	5.18*
				Straying				
1966	3	Cub	NA	1966	2	Cub	NA	3.95*
						Pelican	"	5.39*
1967	3	Clear	BA	1966	3	Cub	"	7.02**
				1967	3	Clear	CBA	4.10*

[a] Legend as in Table 1.

[b] *Significant at $P = 0.05$; **significant at $P = 0.01$.

The average homing times of nonanesthetized fish from Clear and Cub creeks (1966) tagged after tracking from point 2 were 68 and 63 hr greater, respectively, than those of similarly treated fish in the group-tagging experiments. The average homing times of nonanesthetized fish from Clear and Cub creeks (1966) tagged after tracking from point 3 were 49 and 84 hr greater, respectively, than those of similarly treated fish in the group-tagging experiments.

FLOAT-TRACKING STUDIES

Experiments from point 2 — A total of 94 fish was tracked from point 2. The directions of orientation for the experimental groups were as follows: nonanesthetized fish from Clear Creek — east-northeast; controls for blind fish from Clear and Cub creeks — east; nonanesthetized fish from Pelican and

Cub creeks and spawned-out fish from point A — east-southeast; blind fish from Clear Creek and nonspawning fish from point A — southeast; and blind fish from Cub Creek — south (Table 5).

Vector lengths were significantly greater than zero for nonanesthetized fish from Clear Creek and nonspawning fish from point A. Nonsignificant *r* values for other groups of fish may be due to small sample size rather than lack of orientation. Average swimming speeds and *r* values often decreased after the sighting at 0.5 hr after release. Blind fish had smaller *r* values than either the control or nonanesthetized fish. Nonspawning fish oriented as well as or better than any other experimental group of fish.

Mean directions for all groups of spawning fish at the conclusion of the tracking experiments were not significantly different from the home-stream directions except for nonanesthetized fish from Pelican and Clear creeks (Table 5). The only groups whose mean directions after tracking ended differed significantly were nonanesthetized fish from Clear Creek and nonspawning fish (Tables 6, 7). However, when the current direction was used as the zero direction for each fish instead of true North, the mean directions of these two groups were not significantly different. The average speed, mean direction, and vector length for currents were 165 m/hr, 158°, and 0.8076, respectively.

Nonspawning and spawned-out fish had the fastest average swimming speeds of all groups tested (Table 5). Average swimming speeds for other groups of fish were similar to those reported by Jahn (1966) and McCleave (1967).

Mean directions and vector lengths of males and females generally were not different, but females had greater average swimming speeds.

Experiments from point 3 — The total numbers of fish tracked from point 3 in 1966 and 1967 were 59 and 79, respectively. The directions of orientation for fish in the experimental groups in 1966 were as follows: nonanesthetized fish from Clear and Cub creeks and nonspawning fish from points A and B — east-northeast; and spawned-out fish from points A and B — east-southeast (Table 8). Those observed for fish tracked in 1967 were as follows: blind-anosmic fish from Clear Creek — north; controls for blind-anosmic fish from Clear Creek — east-northeast; nonanesthetized fish from Cub Creek — east; nonanesthetized fish, from Arnica Creek — east-southeast; nonanesthetized fish from Hatchery Creek — south-southwest; nonanesthetized fish from Clear Creek tracked in the late afternoon — west-northwest.

Vector lengths (*r*) were significantly greater than zero for nonanesthetized and blind-anosmic fish from Clear Creek and nonspawning fish from points A and B. Nonanesthetized fish from this creek tracked from point 3 during the late afternoon oriented the best of all experimental groups.

Nonanesthetized fish tracked in the late afternoon and blind-anosmic fish from Clear Creek and nonanesthetized fish from Arnica Creek were the only groups whose mean directions differed significantly from the home-stream direction (Table 8). With true North as the zero direction, the mean direction of nonanesthetized fish from Clear Creek tracked in the late afternoon differed

TABLE 5. Mean directions (from true North), vector lengths, swimming speeds, Rayleigh tests, and homestream tests at ½-hr intervals after release for fish tracked from point 2 (1966).

Origin creek	Group[a]	No. tracked	Hr after release	Mean direction (*a*)	Vector length(*r*)	Speeds (*m/hr*)		Rayleigh test[b]	Homestream test[b]
						Range	Avg		
Pelican	NA	9	0.5	125°	0.7797	97–779	434	5.47**	7.02**
		3	1.0	223	0.3730	83–151	111	–[c]	–[c]
		4	1.5	188	0.1575	71–291	139	–[c]	0.63
		3	2.0	293	0.3620	79–199	158	–[c]	–[c]
		10	Term.	114	0.5057	79–779	408	2.55	5.06*
Clear	NA	16	0.5	83	0.3926	20–622	325	2.47	6.28
		14	1.0	58	0.2971	82–519	294	1.24	4.16
		10	1.5	20	0.5509	80–512	213	3.04*	5.51*
		7	2.0	16	0.8651	45–508	171	5.24**	6.06**
		18	Term.	68	0.5445	45–622	323	5.34**	9.80**
Clear	B	8	0.5	137	0.2995	178–968	481	0.72	2.40
		6	1.0	78	0.2863	156–674	345	0.49	1.72
		3	1.5	48	0.5900	131–317	231	–[c]	–[c]
		2	2.0	27	0.4383	120–289	205	–[c]	–[c]
		8	Term.	130	0.3669	120–968	502	1.08	2.94
Clear	CB	8	0.5	69	0.6430	259–977	556	3.31*	5.14*
		5	1.0	32	0.3747	106–558	350	–[c]	1.87
		3	1.5	98	0.1692	129–225	190	–[c]	–[c]
		1	2.0	330	1.0000	189	189	–[c]	–[c]
		8	Term.	94	0.4146	189–977	503	1.38	3.31

(*Continued*)

TABLE 5. Mean directions (from true North), vector lengths, swimming speeds, Rayleigh tests, and homestream tests at ½-hr intervals after release for fish tracked from point 2 (1966). — (*Concluded*)

Origin creek	Group[a]	No. tracked	Hr after release	Mean direction (*a*)	Vector length(*r*)	Speeds (*m/hr*)		Rayleigh test[b]	Homestream test[b]
						Range	Avg		
Cub	NA	14	0.5	228°	0.1781	125–760	367	0.44	2.49
		11	1.0	248	0.2620	36–473	243	0.76	2.88
		8	1.5	319	0.0082	134–272	214	0.00	0.66
		5	2.0	31	0.1792	86–255	181	–[c]	0.90
		14	Term.	121	0.2730	86–760	336	1.04	3.82
Cub	B	6	0.5	109	0.3162	132–569	314	0.60	1.89
		5	1.0	113	0.3623	182–407	272	–[c]	1.81
		4	1.5	57	0.5494	147–484	259	–[c]	2.19
		3	2.0	199	0.2742	124–183	162	–[c]	–[c]
		6	Term.	175	0.2057	124–484	285	0.25	1.23
Cub	CB	6	0.5	99	0.5488	332–966	576	1.80	3.29**
		3	1.0	99	0.7290	217–445	344	–[c]	–[c]
		1	1.5	61	1.0000	168	168	–[c]	–[c]
		1	2.0	57	1.0000	143	143	–[c]	–[c]
		6	Term.	98	0.4666	143–966	509	1.30	2.80
Lake—A,B	NS	22	0.5	133	0.5445	331–962	592	6.52**	–[d]
		7	1.0	181	0.5650	357–650	514	2.23	–[d]
		1	1.5	285	1.0000	419	419	–[c]	–[d]
		1	2.0	301	1.0000	251	251	–[c]	–[d]
		22	Term.	131	0.5849	251–962	586	7.52**	–[d]
Lake—A,B	SO	2	0.5	104	0.8191	533–736	634	–[c]	–[d]
		2	Term.	104	0.8191	533–736	634	–[c]	–[d]

[a] NS = nonspawning; SO = spawned-out; NA = nonanesthetized; B = blind; CB = controls for blind.
[b] *Significant at $P = 0.05$; **significant at $P = 0.01$.
[c] Sample too small for test.
[d] Does not apply.

[c]Sample too small for test.
[d]Does not apply.

TABLE 6. Mean directions and vector lengths at termination of tracking experiments from points 2 and 3, 1966 and 1967, using true North, sun azimuth, and current direction as the zero directions.

				Zero direction as					
				True North		Sun azimuth		Current	
Year	Origin creek	Group[a]	No.	Mean direction	Vector length	Mean direction	Vector length	Mean direction	Vector length
				From point 2					
1966	Pelican	NA	10	114°	0.5057	24°	0.5027	65°	0.2421
	Clear	NA	18	68	0.5445	319	0.3746	297	0.2236
		B	8	130	0.3669	359	0.3677	26	0.6392
		CB	8	94	0.4146	330	0.6493	9	0.2085
	Cub	NA	14	121	0.2730	339	0.3751	49	0.1401
		B	6	175	0.2057	315	0.7240	343	0.4335
		CB	6	98	0.4666	323	0.6041	329	0.8243
	Lake — A,B	NS	22	131	0.5849	358	0.7147	303	0.2377
		SO	2	104	0.8191	323	0.8743	258	0.5446
				From point 3					
1966	Clear	NA	14	63	0.5079	313	0.4992	341	0.3242
	Cub	NA	15	71	0.4156	288	0.4555	30	0.3623
	Lake — A,B	NS	24	75	0.6465	308	0.5217	117	0.5911
		SO	6	123	0.4881	313	0.3278	114	0.4237
1967	Clear	NALA	13	302	0.9235	3	0.9122	5	0.8708
		BA	20	11	0.5130	240	0.4926	32	0.6045
		CBA	14	59	0.3162	296	0.2501	53	0.5212
	Arnica	NA	23	119	0.2679	335	0.3018	18	0.4608
	Cub	NA	5	96	0.6561	257	0.7315	166	0.5393
	Hatchery	NA	4	199	0.5049	82	0.5389	304	0.5049

[a]NALA = nonanesthetized tracked late in the afternoon; BA = blind-anosmic; CBA = controls for blind-anosmic. Others given in legend for Table 5.

TABLE 7. Comparisons of direction test (F) values for tracking experiments using true North, sun azimuth, and current direction as zero directions.

						Direction test (F) value[b] with zero direction as		
Year	Origin creek	Group[a]	vs. Year	Origin creek	Group[a]	North	Azimuth	Current
				From point 2				
1966	Clear	NA	1966	Lake — A,B	NS	7.05*	2.27*	0.01
				From point 3				
1967	Clear	NALA	1966	Clear	NA	27.74**	5.20*	0.65
				Cub	NA	23.33**	9.96**	0.82
				Lake — A,B	NS	58.86**	7.78**	9.53**
					SO	24.50**	2.05	10.25**
			1967	Clear	BA	11.11**	31.10**	2.00
					CBA	13.32**	3.79	4.61
				Arnica	NA	23.47**	1.09	0.34
				Cub	"	35.95**	26.74**	20.89**
				Hatchery	"	11.74**	7.79*	3.78
1967	Hatchery	NA	1966	Clear	"	5.94*	5.93*	0.40
				Cub	"	4.77*	9.42*	2.27
				Lake — A,B	NS	7.60*	6.93*	6.35*
			1967	Hatchery	BA	7.54*	7.53*	3.94
					CBA	4.56*	4.62*	4.47
1967	Clear	BA	1966	Lake — A,B	NS	8.53**	7.35*	2.29
					SO	6.78*	2.09	0.15
			1967	Arnica	NA	9.61**	8.19**	0.32
				Cub	NA	4.99*	0.24	9.92**

[a] Legend as for Table 6.
[b] *Significant at $P = 0.05$; **significant at $P = 0.01$.

significantly from the mean directions of all other groups (Table 7). Almost all of these differences became nonsignificant when the sun azimuth or current direction was used as the zero direction for each fish instead of true North. However, nonspawning fish, and nonanesthetized fish from Cub Creek (1967), still differed significantly from nonanesthetized fish from Clear Creek tracked in late afternoon. The average speed, mean direction, and vector length for currents in 1966 were 402 m/hr, 25°, and 0.6867, respectively, and in 1967 they were 398 m/hr, 319°, and 0.5464, respectively.

Swimming speeds of fish tracked from point 3 (Table 8) were similar to those observed in fish tracked from point 2 (Table 5).

Mean directions and vector lengths of males and females generally were not different, but males had greater average swimming speeds.

Effects of currents — Current affected orientation of many groups of fish, displacing them northward near point 3 and southward near point 2 as they

TABLE 8. Mean directions (from true North), vector lengths, swimming speeds, Rayleigh tests, and homestream tests at termination of tracking experiments from point 3, 1966 and 1967.

Year	Origin creek	Group[a]	No. tracked	Mean direction	Vector length	Speeds (*m/hr*) Range	Speeds (*m/hr*) Avg	Rayleigh test[b]	Homestream test[b]
1966	Clear	NA	14	63°	0.5079	98–787	396	3.61*	7.11
	Cub	NA	15	71	0.4156	118–901	374	2.59	6.23
	Lake — A,B	NS	24	75	0.6465	85–1484	545	10.03**	–[d]
		SO	6	123	0.4881	162–692	458	1.43	–[d]
1967	Clear	NALA	13	302	0.9235	136–959	562	11.08**	12.00**
		BA	20	11	0.5130	33–718	353	5.26*	10.26**
		CBA	14	59	0.3162	213–639	360	1.40	4.43
	Arnica	NA	23	119	0.2679	48–670	349	1.65	6.16**
	Cub	"	5	96	0.6561	185–473	347	–[c]	3.28
	Hatchery	"	4	199	0.5049	26–413	219	–[c]	2.01

[a] NA = nonanesthetized; NS = nonspawning; SO = spawned-out; NALA = nonanesthetized late afternoon; BA = blind-anosmic; CBA = controls for blind-anosmic.

[b] *Significant at $P = 0.05$; **significant at $P = 0.01$.

[c] Sample too small for test.

[d] Does not apply.

swam eastward. Effect of current was demonstrated by the elimination of many significantly different mean directions between groups of fish when the current directions were used as the zero direction for each fish (Table 7).

UNDERWATER PHOTOGRAPHS

No land features were visible on underwater pictures taken from depths of 0.5, 1.0, and 2.0 m at points 2 and 3. Clouds were visible on several of these pictures. Tops of trees were distinguishable on a few pictures taken from depths of 1.0 and 2.0 m 20–80 m from shore. Clouds and trees were visible only on pictures taken when the lake surface was very calm. Even though these images appeared on some photographs, it was not determined whether or not the fish used them for orientation.

TRAINING EXPERIMENTS

Two fish subjected to 5 trials per day for 30 consecutive days using a 100-w light bulb were not trained at the end of this time to use the light source as a reference point (Fig. 2). Three of 6 fish subjected to 10 trials per day for 10 consecutive days using a 300-w light bulb were trained. Two of these tested 30 hr after their normal drilling time showed no compensation in direction for the change in time. Three untrained fish used as controls generally swam away from the light source. Retention of learning was shown by one fish tested 3 weeks after the last drilling session.

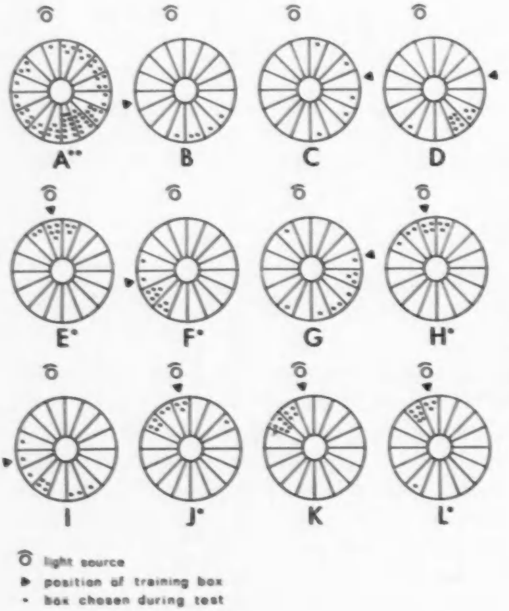

FIG. 2. Results of training experiments using a light source as a reference point for orientation. A — undrilled controls; B, C — fish subjected to 5 trials per day, after 30 days drilling; D, E, F, G, H, I — fish subjected to 10 trials per day, after 10 days drilling; J, K — fish tested 30 hr after last drilling (E and H, respectively); L — fish H tested 3 weeks after the last drilling; * — significant value for normal approximation test at $P = 0.05$; ** — significant value for chi-square test at $P = 0.01$.

DISCUSSION

Significantly fewer anosmic and blind-anosmic fish homed than other groups. This could be due to the lack of olfaction or vision and olfaction, respectively, or to handling and the trauma of injection of material into the olfactory capsules and eyes. Only 6 of 50 anosmic and 2 of 50 blind-anosmic fish homed after they were displaced 5.0 km. McCleave (1967) found that 7 of 50 anosmic (with both olfactory plugs intact) and 25 of 50 blind fish homed. From the results of all these experiments, the combination of olfaction and vision together rather than alone seems important to homing. The importance of olfaction probably increased as the fish neared the homestream but probably was less important in open-water (Hasler, 1966; Brett and Groot, 1963).

No difference was noted in the percentages of homing for anesthetized (control) and nonanesthetized fish. Thus handling during anesthetization and the anesthetic did not affect homing ability. This was also found by McCleave (1967).

Ricker (personal communication) suggested that some straying may be an artifact due to "proving," in which a fish may ascend a "wrong" tributary some distance and then reject it. If a fish was caught in a trap on the "wrong" tributary it was recorded as a stray. "Proving" could occur on Clear and Cub creeks since nearly all average straying times were less than homing times. This was also true for the 1966 data given by McCleave (1967). The fact that more Cub Creek than Clear Creek fish strayed might be due to the trap being closer to the lake on Clear Creek (75 m) than on Cub Creek (150 m). If "proving" did occur a short distance upstream from the mouths of the creeks, then higher straying by Cub Creek fish would be expected. Furthermore, when fish were caught in the traps, they were assumed to be in the homestream. Because of "proving" this may have been an incorrect assumption.

Average homing times were not always directly related to the distance fish were displaced. Those released just outside the stream mouth had the shortest average homing time, but the average homing time for fish displaced 22.0 km from the homestream was shorter than for those displaced 5.0 km away. It is possible that those fish displaced an intermediate distance from the homestream might not determine their positions in the lake before encountering a new set of cues (e.g., shoreline). They might become confused and require additional time to reorient themselves after encountering the new cues, causing a delay in homing time. Those fish displaced farthest from the homestream might determine their positions before encountering more cues and by swimming eastward return fairly directly to an area near the homestream. McCleave (1967) also found an inverse relationship of homing time to distance for fish released in 1966. He suggested that displacement may cause physiological and behavioral changes resulting in delay while the trout begins a new sequence of events leading to migration and spawning.

Other delays could reduce or possibly eliminate any difference in homing time between different groups of fish. McCleave (1967) suggested a delay might occur at the stream mouth, where salmonids are known to congregate before moving upstream. Cope (1956) noted that periodic freshets of cold water caused interruptions in upstream migration of cutthroat trout.

Although homing percentages were similar for fish used in group-tagging experiments and after tracking, the latter had greater homing times. Perhaps the fish became fatigued after towing the floats.

Average swimming speeds and *r* values often decreased after the sighting at 0.5 hr after release for fish tracked from point 2. This might be due to termination of the experiment in a short time when the fish went directly toward shore. Fish swimming away from shore after the first sighting may have tired towing the float. Average swimming speeds were comparable to those reported by Jahn (1966) and McCleave (1967).

The influence of the sun on orientation was shown by fish from Clear Creek (on east side of the lake) that went west-northwest when tracked in the late afternoon, and by those from Arnica Creek (on west side of the lake) and nonspawning fish (from western areas of the lake) that went generally eastward when tracked in the morning. The mean directions of orientation of many groups of fish were closer to 0°, *r* values increased, and significantly different directions of orientation were eliminated when the zero direction was taken as the sun azimuth rather than true North. These experiments and previous ones by Jahn (1966) and McCleave (1967) were confined to relatively short time periods, and the orientation of most fish represents an initial orientation toward the sun. Tracking over longer periods of time might indicate if in swimming toward the sun a bearing might be obtained and later a change in orientation might occur to allow the fish to get home. It might then be determined if trout have the ability to maintain a constant compass direction in the sense of dead reckoning (type II orientation) or have the ability to find home by true navigation involving corrective feedback (type III orientation). Data obtained so far probably are not sufficient to determine which type (if either) may exist in cutthroat trout.

Mean directions of most groups of fish were not significantly different from the homestream direction. The same was true for fish tracked by Jahn (1966). By swimming toward the sun they were also heading toward their homestreams. Fish tracked by McCleave (1967) had mean directions similar to those of this study and to those of Jahn (1966) but significantly different from the homestream direction. The release points used by McCleave were located in the northern portion of the lake.

Due to the small number of training experiments that were conducted, no relationship could be established with the field data concerning orientation. However, cutthroat trout were able to use a single light source for the reference point in orientation.

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REFERENCES

BATSCHELET, E. 1965. Statistical methods for the analysis of problems in animal orientation and certain biological rhythms. Am. Inst. Biol. Sci., Washington, D.C. 57 p.

BENSON, N. G. 1961. Limnology of Yellowstone Lake in relation to the cutthroat trout. U.S. Fish Wildlife Serv. Res. Rept. 56: 1–33.

BRETT, J. R., AND C. GROOT. 1963. Some aspects of olfactory and visual responses in Pacific salmon. J. Fish. Res. Bd. Canada 20: 287–303.

COPE, O. B. 1956. Some migration patterns in cutthroat trout. Proc. Utah Acad. Sci. 33: 113–118.

GREENWOOD, J. A., AND D. DURAND. 1955. The distribution of length and components of the sum of n random unit vectors. Ann. Math. Statist. 26: 233–246.

HASLER, A. D. 1966. Underwater guideposts: homing of salmon. Univ. Wisc. Press, Madison, Milwaukee, and London. 155 p.

HASLER, A. D., R. M. HORRALL, W. J. WISBY, AND W. BRAEMER. 1958. Sun-orientation and homing in fishes. Limnol. Oceanog. 3: 353–361.

JAHN, L. A. 1966. Open-water movements of the cutthroat trout (*Salmo clarki*) in Yellowstone Lake after displacement from spawning streams. J. Fish. Res. Bd. Canada 23: 1475–1485.

MCCLEAVE, J. D. 1967. Homing and orientation of cutthroat trout (*Salmo clarki*) in Yellowstone Lake, with special reference to olfaction and vision. J. Fish. Res. Bd. Canada 24: 2011–2044.

MCCLEAVE, J. D., L. A. JAHN, AND C. J. D. BROWN. 1967. Miniature alligator clips as fish tags. Progressive Fish-Culturist 29: 60–61.

PLATTS, W. S. 1959. Homing, movements, and mortality of wild cutthroat trout (*Salmo clarki* Richardson) spawned artificially. Progressive Fish-Culturist 21: 36–38.

STEEL, R. G. D., AND J. H. TORRIE. 1960. Principles and procedures of statistics, with special reference to the biological sciences. McGraw-Hill, New York. 481 p.

STEPHENS, M. A. 1962. Exact and approximate tests for directions. I. Biometrika 49: 463–477.

WALLS, G. L. 1942. The vertebrate eye and its adaptive radiation. Cranbrook Press, Bloomfield Hills, Mich. 785 p.

WATSON, G. S., AND E. J. WILLIAMS. 1956. On the construction of significance tests on the circle and the sphere. Biometrika 43: 344–352.

Effect of Finclipping on Survival of Sockeye Salmon (*Oncorhynchus nerka*)

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WEBER, DOUGLAS, AND ROY J. WAHLE. 1969. Effect of finclipping on survival of sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada 26: 1263–1271.

A finclip and a chemical (tetracycline) mark were applied, in similar numbers, to hatchery-reared fingerling sockeye salmon (*Oncorhynchus nerka*). In mark-sampling of returning adults 3 and 4 years after release from the hatchery, numerical comparison of fish with the two marks showed that fewer finclipped fish returned than expected. It was demonstrated that the tetracycline mark did not affect survival and that the difference between returns of fish with the two marks originated in mortality caused by finclipping. The mortality attributed to finclipping was estimated as 39%.

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INTRODUCTION

FINCLIPPING IS WIDELY USED for marking fish for population estimates. In these estimates the extent of fin regeneration and effects of clipping on survival must be considered. Fin regeneration is typically estimated by observations on held fish, but evaluation of long-term mortality induced by finclipping offers a more difficult problem. Few experiments have allowed valid estimates of mortality from finclipping in open populations (i.e., where immigration into or emigration from the population under study is possible), primarily because of the lack of an additional mark — neither beneficial nor detrimental to survival — with which a finclip may be compared.

Tetracycline antibiotic was applied in the present study as a mark that would not affect the growth or survival of the fish (Weber and Ridgway, 1967). When administered orally, parenterally, or by immersion, tetracycline is deposited permanently on the growing surface of skeletal tissue. The resulting mark is detected as a fluorescent yellow band upon exposure of the marked bone to ultraviolet illumination.

METHODS

FISH USED

Hatchery-reared sockeye salmon (*Oncorhynchus nerka*) of the 1961 year-class (year of egg deposition) were used in this investigation. These fish were progeny of salmon that spawn in the Lake Wenatchee drainage and whose eggs were removed and hatched at the Leavenworth National Fish Hatchery (Fig. 1). The fry and fingerlings were reared at the hatchery until autumn 1962 and then released in Lake Wenatchee. They lived in the lake until the following spring,

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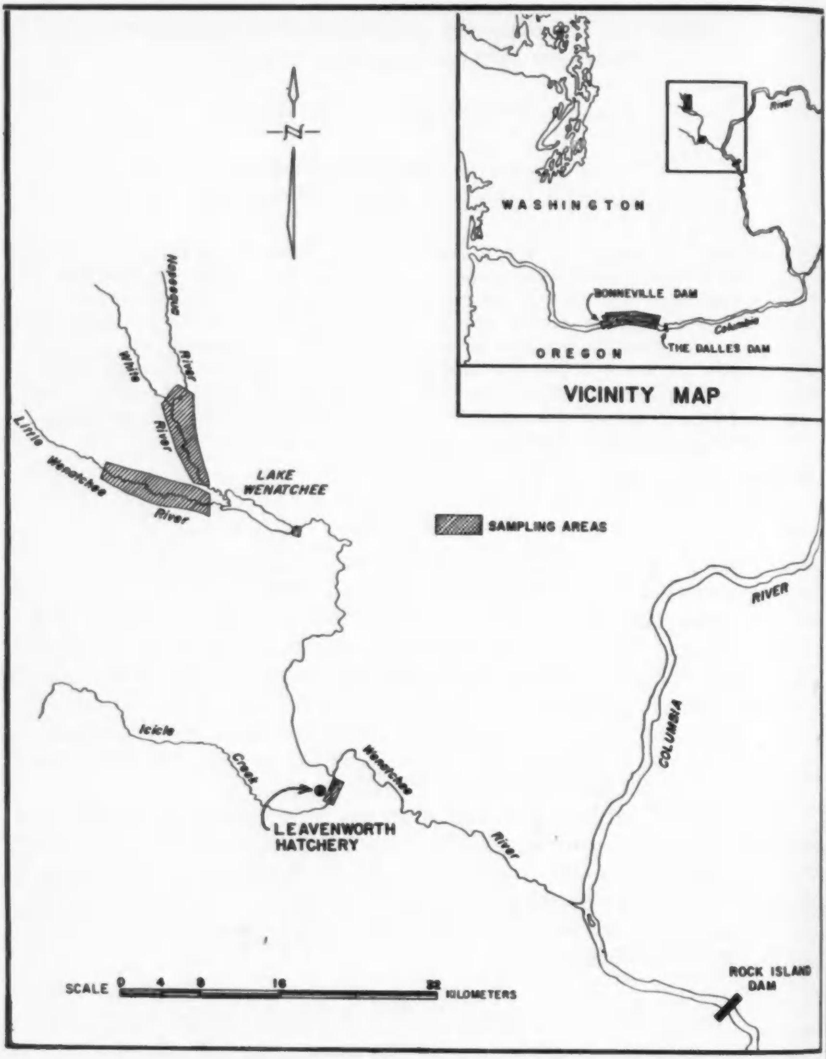
N
VICINITY MAP
WASHINGTON
OREGON
BONNEVILLE DAM
THE DALLES DAM
Columbia
River
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Napeequa River
White River
Little Wenatchee River
LAKE WENATCHEE
RIVER
COLUMBIA
Icicle Creek
LEAVENWORTH HATCHERY
Wenatchee River
ROCK ISLAND DAM
SCALE 0 4 8 16 32 KILOMETERS

FIG. 1. Sampling areas for recovery of marked sockeye salmon.

when they migrated into the Columbia River and thence into the Pacific Ocean, along with naturally spawned sockeye salmon smolts. Most sockeye salmon in the Wenatchee drainage return to spawn after 2 years of oceanic life as 4-year-olds; the rest return in the 5th year of life.

FINCLIPPING AND TETRACYCLINE MARKING

In July 1962 the Leavenworth Hatchery had 30 ponds that contained fingerling sockeye salmon of the 1961 year-class. We removed one-third of the fingerlings in each pond by a three-pocket sampler with one pocket closed and then finclipped them. (This sampler was adapted from one described by Hewitt and Burrows, 1948.) After the fish were marked by removing the adipose fin and part of the left maxillary (AD-LM), they were returned to their respective ponds.

In August, 2 g/kg body weight of oxytetracycline hydrochloride were fed to the fingerlings in 10 of the 30 holding ponds over 3½ consecutive days. A random sample of 550 fish taken 2 months later from the 10 ponds indicated that all the fingerlings that were fed tetracycline were marked.

ESTIMATES OF RELEASE

Hatchery personnel released the fingerling sockeye salmon into Lake Wenatchee in mid-October. At release the entire hatchery production was estimated with a 10-pocket pie-shaped sampler, which had one pocket closed to retain 10% of the fish. The fish retained were resampled; 6733 from the ponds with fingerlings marked with tetracycline and 12,084 from the 20 remaining ponds were examined to determine the percentage of finclipped fish. From these numbers of fish and the estimated proportion retained by the sampler, the numbers of fingerlings in each category at time of release were calculated (Table 1).

TABLE 1. Estimated numbers of marked and unmarked sockeye salmon fingerlings released from Leavenworth Hatchery into Lake Wenatchee in 1962.

Type of mark	No. of fingerlings
Clipped fins	369,752
Tetracycline	425,859
Tetracycline and clipped fins	201,974
Unmarked	779,616
Total	1,786,201

Sampling and release of the sockeye salmon fingerlings required 10 days. In each group released, one-third of the fingerlings were tetracycline-marked (some had double marks — tetracycline plus finclip). This precaution was taken in the event of unusual mortality in any single release group.

In the 2 months between tetracycline-marking and release, mortalities of marked and unmarked fingerlings were similar in all of the 30 ponds. One aspect of the post-planting history of the fish caused some concern, however. Even though the fingerlings of all categories were disease-free in the hatchery, the antibiotic used for marking some of them might have been retained in their tissues in sufficient quantity to continue acting as a therapeutic agent after release.

In another study (unpublished) the bioassay of tissue levels of oxytetracycline after a total dose of 1 g/kg of body weight given to fingerling chinook salmon (*Oncorhynchus tschawytscha*) over 4 days showed nondetectable amounts (less than 0.1 mg/g) 20 days after withdrawal. Thus, even though the total tetracycline dosage given the sockeye salmon was double the amount given the chinook salmon, the sockeye salmon fingerlings were held 2 months before they were released after administration of the antibiotic; the antibiotic residue, if any, was thus unlikely to be effective as an antibiotic. Furthermore, Anderson et al. (1959) demonstrated in rabbits that, "Although there is a persistence of tetracycline in bone . . . the therapeutic action at least against certain common pathogens appears to be limited to within 96 hours after the cessation of the antibiotic medication."

The mortality of finclipped fish from handling and post-operative infection would have been complete within several days after clipping, or 3 months before release. Hatchery records of daily mortality gave evidence of differential mortality between finclipped and unclipped fish in only the first 2 days after clipping. Thus, the estimates of population composition in Table 1 would not have been modified after release by either a beneficial effect of tetracycline antibiotic or delayed mortality from the actual process of finclipping.

FIN REGENERATION

During the enumeration before release at Leavenworth Hatchery in 1962, 200 AD-LM clipped sockeye salmon fingerlings were removed and kept for periodic observations on fin regeneration. In May 1963, when the smolts from the hatchery population were migrating to the ocean, the clipped fish retained at Leavenworth Hatchery were moved to saltwater holding facilities at Bowman Bay on Puget Sound.

The clipped sockeye salmon, after 2 years at Bowman Bay, showed no regeneration of the adipose fin, but the left maxillary regenerated in 8% of the fish. Similar experiments with 300 sockeye salmon of the 1960 and 1962 year-classes also indicated no regeneration of the adipose fin. The occurrence of naturally missing adipose fins (recorded during finclipping) was less than 0.01%.

Records for 194 finclipped adult sockeye salmon of the 1961 year-class that were recovered in the fishery and on the spawning grounds showed a 6% regeneration of the left maxillary — not significantly different from the percentage observed in fish held in ponds.

Because adipose fin regeneration was not evident and because the occurrence of naturally missing adipose fins is so low, we assume confidently that all clipped sockeye salmon in our samples were detected and that they all represented individuals from the original hatchery population. In our analysis the recovered "adipose-only" clipped sockeye salmon are grouped with, and considered as, AD-LM clipped fish.

RECOVERY OF MARKED SOCKEYE SALMON

The sockeye salmon in this study were recovered at four different intervals after their release from Leavenworth Hatchery (Table 2): in the spring of 1963 as smolts migrating out of Lake Wenatchee; in the summer of 1965 as returning adults in the Indian fishery between Bonneville and The Dalles dams on the Columbia River; in the fall of 1965 on the spawning grounds; and in the fall of 1966 on the spawning grounds.

TABLE 2. Numbers of marked sockeye salmon recovered at different locations after their release into Lake Wenatchee in 1962.

Year	Location of recovery	Sample size	No. of fish with clipped fins only	No. of fish with tetracycline mark only	No. of fish with clipped fins and tetracycline mark
1963	Lake Wenatchee	2,020	493	436	255
1965	Indian fishery	1,755	17	43	7
	Spawning grounds	4,075	72	145	45
	Combined areas	5,830	89	188	52
1966	Spawning grounds	1,938	10	16	7
Total		15,618	681	828	366

Smolts were sampled at the outlet of Lake Wenatchee (Fig. 1) from mid-April to the end of May in 1963. We collected samples during darkness with eight permanently set fyke nets. The nets were arranged in two rows so that the four downstream nets covered the spaces between the four upstream nets. The nets screened about one-half the stream width and one-half its depth

The fyke nets were emptied each morning and a sample of smolts taken with a three-pocket net with one pocket closed. After each sampled fish was measured, weighed, and checked for finclips, an anterior rib was removed for examination of a tetracycline mark.

We sampled 4-year-old adult sockeye salmon returning in 1965 at two locations: the Indian fishery on the Columbia River between Bonneville Dam and The Dalles Dam and on the spawning grounds above Lake Wenatchee and on Icicle Creek. Sampling was carried out during the entire fishing and spawning seasons.

Sockeye salmon from the Indian fishery on the Columbia River were examined at fish-buying stations and canneries in Washington and Oregon. Either the entire day's catch or every fourth fish passing through the processing line was sampled. The sample fish was checked for clipped fins before dressing, a vertebra was removed after dressing, and the vertebral centrum was examined for the presence of a tetracycline mark.

Carcasses of spawned-out fish were collected on the spawning grounds of Icicle Creek and the Little Wenatchee and White rivers above Lake Wenatchee. Each carcass encountered on the shore or stream bed was sexed, measured, and checked for clipped fins, and a vertebral centrum was removed.

For 1965 (Table 2) the relative numbers of recoveries of the three mark types did not differ significantly between recovery areas ($\chi^2 = 2.34$, 2 df); thus the 1965 Indian fishery and spawning ground samples were combined and treated as a single recovery unit.

Because the Indian fishery on the Columbia River did not operate in 1966, samples of returning 5-year-old adult sockeye salmon were taken only in tributaries of Lake Wenatchee and in Icicle Creek.

Age analysis (from oceanic growth rings on the vertebrae) of adult sockeye salmon collected in 1965 indicated that 95% of the 5-year-old fish were over 52 cm in fork length. To eliminate 4-year-old fish (not marked with tetracycline), vertebral centra were removed in 1966 only from carcasses of spawned-out fish 52 cm and greater in fork length.

SURVIVAL OF TETRACYCLINE-MARKED FISH

As the first step in our analysis we tried to determine what effect tetracycline in the skeletal system may have on survival. This effect may be estimated by comparing individuals with a double mark (tetracycline plus finclips) and those with finclips only. Because both groups in this comparison are finclipped, this evaluation shows only the effect of tetracycline. At the time of release from Leavenworth Hatchery, 35.3% of the fingerlings with finclips were also marked with tetracycline. A significant variation at recovery from this percentage of double marks at release would indicate an effect of tetracycline on survival.

The data (Table 3) show that tetracycline had no effect on survival of the fish either during their 7 months as fingerlings in Lake Wenatchee or during their 2 or 3 years at sea.

Any loss of the tetracycline mark itself or defects in our detection of the fluorophore would have shown up as tetracycline-induced mortality. Our findings and the study of Weber and Ridgway (1967) make us confident the tetracycline mark is permanently retained without diminution.

SURVIVAL OF FINCLIPPED FISH

The data of the preceding section show that a tetracycline mark is neither beneficial nor detrimental to survival and thus may be used to judge the

TABLE 3. Relation between number of fish with double marks (tetracycline plus finclips) and number of fish with clipped fins at time of release in 1962 and times of recovery in 1963, 1965, and 1966.

Year and life stage	Total no. of fish with double marks or clipped fins only	Percentage with double marks	95% confidence intervals
1962, fingerlings	571,726	35.3	34.1–36.6
1963, smolts	748	34.1	30.6–37.6
1965, adults	166	38.6	30.6–46.6
1966, adults	28	28.6	14.8–42.3
1965 and 1966, combined adults	194[a]	37.1	29.8–44.4

[a] Thirty-six of these finclipped fish were collected in a concurrent, but separate, study on population abundance of spawning sockeye salmon. The remainder of the fish represented in this report were randomly sampled.

effect of finclipping on survival. In this section we consider estimates of mortality over the period from release as fingerlings in 1962 through return as adult fish in 1965 and 1966.

In Table 4 we show the relation between tetracycline marks only and finclips only at release and return. At each recovery interval the percentage of finclipped fish within this grouping was lower than would be expected if survival were equal in both groups. The combined 1965 and 1966 percentage of adult returns of finclipped fish was 30% less than the percentage of finclipped fingerlings released.

TABLE 4. Relation between number of fish with tetracycline marks only and number of fish with clipped fins only at time of release in 1962 and times of recovery in 1965 and 1966 (see Tables 1 and 2 for basic data).

Year and life stage	Total no. of fish with tetracycline marks only or clipped fins only	Percentage with clipped fins only
1962, fingerlings (release)	795,611	46.5
1965, adults	277	32.1
1966, adults	26	38.5
1965–66, combined adults	303	32.7

The change in percentage for finclipped sockeye salmon between release and return, as shown in Table 4, may also be expressed in terms of mortality attributed to the AD-LM clip combination. Estimation of mortality from clipping was based on a comparison of the numbers doubly marked with those with tetracycline marks only at release and recovery, and was determined by the equality:

$$\frac{R_{TC}}{R_T} = \frac{N_{TC}e^{-(m+t+c)}}{N_T e^{-(m+t)}}$$

where: R_{TC} = number recovered with double marks; R_T = number recovered with tetracycline marks only; N_{TC} = number released with double marks; N_T = number released with tetracycline marks only; and m, t, c, = instantaneous coefficients of mortality — natural, induced by tetracycline, and induced by finclipping, respectively. Rearranging the foregoing equality to solve for survival of finclipped fish:

$$e^{-c} = \frac{R_{TC}(N_T)}{R_T(N_{TC})} .$$

Values obtained by this equation for mortality ($1-e^{-c}$) caused by finclipping sockeye salmon from time of release as fingerlings from Leavenworth Hatchery in 1962 to time of return as adult fish in 1965 and 1966 are given in Table 5.

TABLE 5. Estimates of mortality from finclipping ($1-e^{-c}$) for sockeye salmon from time of release in 1962 to times of recovery in 1965 and 1966.

Year and location of recovery	No. of fish with tetracycline marks only	No. of fish with double marks	Mortality due to finclipping ($1-e^{-c}$)	Instantaneous mortality (i)
1965, combined areas	188	52	0.4168	0.5386
1966, spawning grounds	16	7	0.0775	0.0801
1965 and 1966, combined	204	59	0.3902	0.5011[a]

[a]Weighted instantaneous mortality value based on 3 years out equals $a_{i1965}+b_{i1966}$, where: $a = \frac{188}{204}$; $b = \frac{3}{4}\left(\frac{16}{204}\right)$.

According to our model, mortality as a result of finclipping in the 1966 recoveries should be equal to or greater than that shown in the 1965 recoveries. The apparently lower mortality indicated by finclipped adults returning in 1966 than by those returning in 1965 can reasonably be attributed to the small 1966 sample, because the difference in length between spawners marked by finclipping and those marked by tetracycline (Fig. 2) was too small to cause selection in sampling on the spawning grounds.

Variation also occurred in mortality attributed to finclipping within the 1965 samples. Even though the basic data presented in Table 2 indicate no significant difference in the relative numbers of fish in the three mark categories between areas, it should be noted that the calculated mortality from finclipping ($1-e^{-c}$) in samples from the Indian fishery was 0.6568. Thus, the 1965 combined area mortality of 0.4168 (Table 5) may be high because of selectivity of the fishery, especially if the finclipped fish were smaller and managed to slip through the gillnets more readily than the tetracycline-marked fish. Length and sex measurements taken at the time of sampling the Indian fishery were insufficient to show if this type of selectivity existed. Any fishery selectivity of this nature

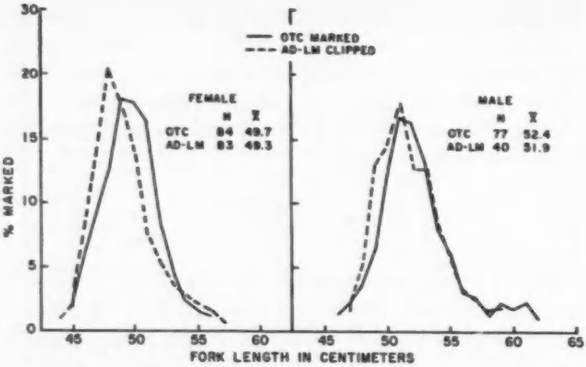

FIG. 2. Length-frequency distributions, by sex, of tetracycline-marked (OTC) and finclipped (AD-LM) adult sockeye salmon sampled on the spawning grounds in 1965 and 1966.

would be partly compensated, however, by a greater probability of recovering proportionately more finclipped fish on the spawning grounds. Combined returns of 1965 and 1966 recoveries show that finclipped fish suffered a 39% greater mortality than those marked with tetracycline.

The results from smolt samples in 1963 present an inconsistency concerning differential survival between fish marked with tetracycline and by finclipping at each recovery stage. Of the total number of fish with tetracycline marks only or clipped fins only, at the time of migration from Lake Wenatchee (Table 2), the percentage that had clipped fins was 53.1%. This percentage indicates a greater survival of finclipped fish than expected from the release percentage of 46.5% (Table 4). Similarly, a survival estimate (e^{-c}) for the finclipped smolts was greater than one, indicating a negative mortality.

A possible explanation for the recovery of finclipped smolts in greater numbers than expected is based on differential recovery in relation to size at time of smolt migration between those marked with tetracycline and those finclipped. As shown in Fig. 3A, the sizes of smolts recovered decreased progressively for both methods of marking during the sampling period. This

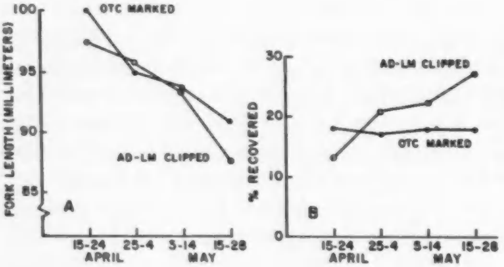

FIG. 3. Recoveries of tetracycline-marked (OTC) and finclipped (AD-LM) sockeye salmon smolts at time of migration from Lake Wenatchee in 1963: (A) average fork lengths of smolts by 10-day periods; (B) percentages of each type of mark in daily samples averaged over 10-day periods.

decrease in size correlates with a progressive increase in the ratio of finclipped smolts to tetracycline-marked smolts (Fig. 3B), indicating that the percentage of the smaller smolts that were finclipped was higher than those marked with tetracycline. In addition, there is good evidence from previous sockeye smolt migration studies conducted at Lake Wenatchee that the fyke nets used in sampling were selective for smaller fish (Don Craddock, personal communication). The combined size differential and gear selectivity would account for the greater proportion of finclipped smolts recovered.

ACKNOWLEDGMENTS

R. A. Fredin, D. D. Worlund, and J. M. Shelton of the Bureau of Commercial Fisheries assisted in the design and analysis. We appreciate the cooperation and assistance of personnel of the Bureau of Sport Fisheries and Wildlife at Leavenworth National Fish Hatchery, and members of the Columbia River investigation section of the Fish Commission of Oregon.

REFERENCES

Anderson, Russell J., Jr., Albert B. Ferguson, and Abraham T. Brande. 1959. Bacteriostasis of tetracycline deposited in bone. Surg. Gynecol. Obstet. 108: 65–67.

Hewitt, George S., and Roger E. Burrows. 1948. Improved method for enumerating hatchery fish populations. Progressive Fish-Culturist 10: 23–27.

Weber, Douglas, and George J. Ridgway. 1967. Marking Pacific salmon with tetracycline antibiotics. J. Fish. Res. Bd. of Canada 24: 849–865.

Comparisons of Food of Cod and Haddock in the Gulf of St. Lawrence and on the Nova Scotia Banks

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Changes in diet of cod (*Gadus morhua*) with increasing size were similar in the southern Gulf of St. Lawrence, Sydney Bight, Banquereau, and Western and Emerald banks. Small cod fed mainly on crustaceans and changed to a fish diet as they grew larger. Differences in species taken within the food groups seemed to depend on relative availability of the prey species. When cod reached sizes (ca. 50 cm) where they subsisted mainly on a fish diet, their main prey in the Gulf of St. Lawrence was herring (*Clupea harengus*) and on the Nova Scotia Banks it was sand launce (*Ammodytes americanus*). There were some seasonal differences in food eaten within areas and by depth.

In the Western and Emerald bank areas, cod stomachs contained more food than haddock (*Melanogrammus aeglefinus*), up to two or three times as much for the large size-groups. Cod generally fed more heavily on pelagic crustaceans and fish whereas haddock took benthic animals, including annelid worms, molluscs, and echinoderms. When pelagic prey, particularly fish, were available to cod, there was the least overlap in food species taken by cod and haddock in the same area.

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INTRODUCTION

GROUNDFISH SURVEY CRUISES have been carried out by the Fisheries Research Board of Canada, St. Andrews, N.B., since 1958 on the banks off Nova Scotia and in the Gulf of St. Lawrence. The usual purpose was to study abundance, sizes, ages, maturities, and feeding habits of the principal groundfish species.

In this study, accumulated information on the food of cod and haddock taken on CGS *A. T. Cameron* and M.V. *Harengus* from 1958 to 1963 is analysed. The food items of cod in four areas are compared first. A further section describes the food of cod and haddock caught in the same tows in one area. The uses of the available food supply by the two species in the area where both are fairly abundant (Division 4W, Fig. 1) are compared. The investigation complements previous studies by Corbeil (1954) and Powles (1958) of cod food in the Gulf of St. Lawrence, and by Homans and Needler (1944) of haddock food on Nova Scotia Banks, by comparison of data from other areas, between species, by length group, by season, and by depth.

MATERIALS AND METHODS

A total of 20,340 cod stomachs that contained food (Table 1) were examined from an area between Orphan Bank in the Gulf of St. Lawrence and Emerald Bank off Nova Scotia (Fig. 1),

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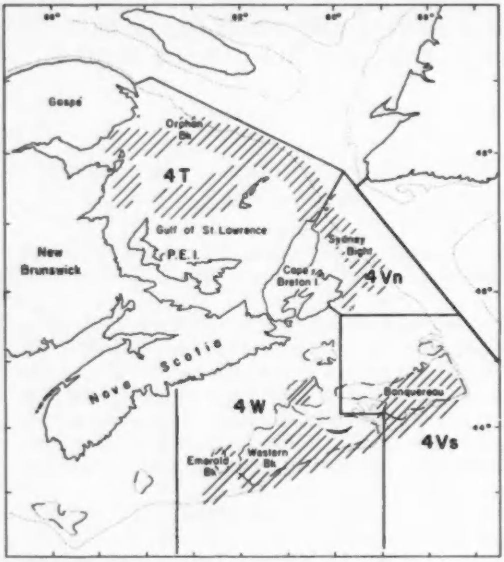

FIG. 1. Fishing banks and ICNAF divisions referred to in the text. Fishing locations are cross-hatched.

as were 4710 haddock stomachs from the Western and Emerald bank areas. All catches were taken by otter trawl using a 1⅛-inch (29-mm) mesh liner in the codend. The *A. T. Cameron* fished a #41 and the *Harengus* a #36 Yankee trawl as described by Jean (1964). Standard procedures for examination and sampling of the catches were initiated in 1958. The data obtained were punched on IBM cards after each cruise, to facilitate summarization.

Stomach contents were examined at sea in conjunction with age–length sampling. Food items were recorded by species, family, class, or phylum, depending on the ease of recognition. Volumes were visually estimated in cubic centimeters using either a series of plasticine models of various volumes or displacement of water in a graduated cylinder as a guide. When more than one item appeared in the same stomach, the proportions of the total were estimated as parts of 10 and the actual volume was calculated later.

A number of observers were involved in the cruises, and it was found by experiment that some tended to overestimate total volumes in cubic centimeters whereas others underestimated. For this reason, estimated volumes may differ from the actual volumes. However, the differences should be consistent over the size and depth ranges and should have little effect on the percentage by volume of food composition since the estimation of parts of 10 was consistent among observers.

Preliminary analyses indicated that the relative importance of various items of food changed significantly with size of cod but less with haddock. Area, depth, and seasonal comparisons showed lesser but still significant differences in the cod diet. Per cent occurrence of food items was not used in this study as some items occurred often but contributed little to the total volume.

For comparison of data by sizes, we divided the sampled cod and haddock into four length-groups: under 30 cm (<30 cm); 30–49 cm; 50–69 cm; and 70 cm and longer (70+ cm). Also, food items from cod and haddock of two depth ranges were compared: from less than 50 fath (91 m) and from 50 fath and deeper. The seasonal divisions used were January–April, May–August, and September–December.

The area boundaries used (Fig. 1) follow the International Commission for the Northwest Atlantic Fisheries (ICNAF) division boundaries and enclose four Divisions within which the following fishing areas are found: southwestern Gulf of St. Lawrence (4T); Sydney Bight (4Vn); Banquereau (4Vs); and Western and Emerald banks (4W).

TABLE 1. Numbers of cod and haddock stomachs examined containing food, by depth, length of fish, season, and area, 1959–62.

ICNAF Division	Season	Depth (<50 *fath*)				Depth (50+ *fath*)				Total
		Length of fish (*cm*)				Length of fish (*cm*)				
		<30	30–49	50–69	70+	<30	30–49	50–69	70+	
					Cod					
4T	Jan.–Apr.	233	117	64	15	265	522	445	291	1,952
4T	May–Aug.	214	1,006	863	254	191	845	813	234	4,420
4T	Sept.–Dec.	668	2,259	1,091	181	171	1,082	856	147	6,455
4Vn	Jan.–Apr.	50	100	74	18	149	466	523	402	1,782
4Vs	"	30	98	123	91	153	386	329	157	1,367
4W	"	252	310	500	323	216	378	427	335	2,741
4Vn	May–Aug.	27	129	126	46	41	266	339	134	1,108
4W	May–Aug.	65	157	119	27	17	48	65	17	515
					Haddock					
										20,340
4W	Jan.–Apr.	164	799	633	–	713	1,525	876	–	4,710

STOMACH CONTENTS OF COD

Comparison by Area and Fish Size

The principal food groups of cod in each of the areas were fish, crustaceans, annelids, molluscs, and echinoderms (Table 2). There were some small quantities (less than 5% of the total volume) of ctenophores, coelenterates, poriferans, protochordates, chaetognaths, and unidentified eggs. In the figures (e.g. Fig. 4), these were combined and labelled "others." A small quantity of unidentified food items and nonfood items such as stones, sand, and mucus, were combined and listed as "unidentified." The first comparisons were by food groups as listed above and, after this, the relative amounts of major items within important groups were examined. The data were separated into those from depths of less than 50 fath and those from 50+ fath.

The period January–April was the only season when fishing was carried out in all four areas surveyed. A number of similarities were apparent in the cod diet from the four areas at that time of year (Fig. 2). One was the high percentage volume of crustaceans (60–80% of the total volume) consumed by cod in the 0–29-cm range. A gradual decrease in the amount of this type of food with increase in size of cod and a corresponding increase in fish items were evident. In all areas but one, crustaceans and fish together made up more than 80% of the total volume. The exception was Division 4Vn, where relatively small quantities of fish items were found in all cod in shoal water (<50 fath). Very few large cod (50+ cm) appeared in the catches in this location.

Annelids, mainly polychaete worms, were found in cod stomachs in all the areas. They were more abundant in the diet of small cod than of large cod. However, they usually made up less than 10% of the total volume even in the small cod. Again, the exception was in Division 4Vn where annelid worms made up 28% of the total in the 0–29-cm cod in shoal water.

Molluscs and echinoderms were not abundant in the diet of small cod but increased in prominence in the cod over 50 cm, especially in divisions 4Vn and 4Vs.

Identification of species within the phyla Mollusca and Echinodermata was not often complete. However, the major portion of the molluscs were clams (Pelecypoda) and the major portion of the echinoderms were brittle stars (Ophuroidea) for all sizes of cod. Items that occurred less frequently from the phylum Mollusca were scallops and cockles (Pelecypoda), whelks (Gastropoda), and squids (Cephalopoda), and from the phylum Echinodermata, sea urchins (Echinoidea), and sea cucumbers (Holothurioidea).

"Others" and "unidentified" together made up a small proportion of the total food consumption. There was one unusual item in the data, an abundance of arrow worms (*Sagitta*) in cod under 30 cm in Division 4Vs in deep water.

Comparison By Seasons within Areas

In the Gulf of St. Lawrence (Fig. 3) the relative importance of particular food items for each of the size-groups was fairly consistent throughout the

TABLE 3. Percentage volumes of various items in stomachs of cod and haddock, by size and depth, taken in Division 4W, January–April 1959–62. Asterisks (*) indicate small quantities of less than 0.5%.

Food item	0–29 cm				30–49 cm				50–69 cm				70+ cm	
	<50 fath		50+		<50		50+		<50		50+		<50	50+
	Had.	Cod	Had.	Cod	Had.	Cod	Had.	Cod	Had.	Cod	Had.	Cod	Cod	only
Fish														
Unidentified	5	4	6	14	4	10	3	18	9	7	9	18	10	42
Haddock & cod (Gadidae)	–	–	–	–	–	–	*	–	–	–	2	*	3	9
Silver hake (*Merluccius bilinearis*)	–	–	–	7	–	–	1	*	–	–	1	21	–	7
Redfish (*Sebastes* spp.)	–	–	*	1	–	–	*	1	–	–	–	*	–	14
Flounders (Pleuronectidae)	–	–	*	–	*	–	–	1	*	*	*	1	5	3
Herring (*Clupea harengus*)	–	–	–	–	–	–	*	–	2	1	5	12	–	–
Argentines (*Argentina silus*)	–	–	–	–	1	–	6	1	–	–	6	–	–	–
Alewives (*Alosa pseudoharengus*)	–	–	–	–	–	–	–	–	–	–	–	3	–	1
Sand launce (*Ammodytes americanus*)	–	5	–	4	9	47	*	2	21	76	*	1	75	3
Sculpins (Cottidae)	–	–	–	–	–	–	–	3	–	–	*	*	*	–
Blennies & shannies (Stichaeidae)	–	–	1	*	*	*	–	1	–	*	*	*	–	2
Total	5	9	8	27	14	58	11	28	32	84	25	59	93	82
Crustaceans														
Unidentified	3	4	3	1	1	*	2	*	*	*	1	*	–	*
Decapoda	2	2	6	11	*	2	3	6	*	1	1	2	*	*
Pandalidae	8	12	8	19	1	1	12	10	1	*	3	8	*	1
Crabs (Brachyura & Paguridae)	2	2	1	3	1	8	3	3	2	1	3	*	–	*
Euphausiidae	3	35	1	17	2	6	4	36	1	3	1	16	*	2
Mysidae	3	2	*	1	*	–	*	*	–	*	*	*	–	*
Amphipoda	12	11	8	7	17	4	6	1	7	*	2	1	*	*
Caprellidae	3	–	–	–	*	–	–	–	–	–	–	–	–	–
Copepoda	–	4	1	*	*	–	*	–	–	–	–	–	–	–
Cumacea	–	–	*	–	*	–	*	–	–	–	–	–	–	–
Isopoda	1	–	*	–	*	–	*	–	*	–	*	*	–	–
Crangonidae & Hippolytidae	–	1	2	4	–	–	1	*	*	*	2	*	–	–
Total	37	72	30	62	22	22	32	56	11	5	13	27	1	3

(*Continued*)

TABLE 2. Percentage volumes of various items in stomachs of cod and haddock, by size and depth, taken in Division 4W, January–April 1959–62. Asterisks (*) indicate small quantities of less than 0.5%. — (*Concluded*)

	0–29 cm				30–49 cm				50–69 cm				70+ cm	
	<50 fath		50+		<50		50+		<50		50+		<50	50+
Food item	Had.	Cod	Had.	Cod	Had.	Cod	Had.	Cod	Had.	Cod	Had.	Cod	Cod	only
Annelids														
Unidentified	14	4	10	*	7	–	8	*	5	*	7	*	–	*
Polychaete worms	3	5	10	2	8	2	8	1	7	*	10	1	*	*
Sea mouse (*Aphrodite*)	–	–	–	–	*	–	*	*	*	–	2	–	–	*
Mud worms (Gephyrea)	–	–	–	–	–	–	–	*	–	–	–	–	–	*
Tube worms (unidentified)	–	–	–	–	1	–	*	*	*	–	*	–	–	–
Total	17	9	20	2	17	2	16	1	12	*	19	1	*	2
Molluscs														
Unidentified	*	*	1	–	1	*	1	*	2	*	2	*	*	*
Gasteropoda	*	–	1	–	2	*	2	2	2	1	2	1	*	*
Pelecypoda	*	–	1	–	1	–	1	*	1	*	1	*	*	*
Clams (Teleodesmacea)	*	1	*	1	3	10	1	6	3	6	2	6	3	9
Scallops (Pectinidae)	–	–	–	–	–	–	*	–	–	–	*	–	–	–
Cockles (Cardiidae)	*	–	1	–	*	–	1	–	–	–	*	–	–	–
Nutshells (Nuculidae)	–	–	–	–	*	–	*	–	*	–	*	–	–	–
Sea slugs (Nudibranchia)	–	–	–	–	–	–	–	–	–	–	*	–	–	–
Squids (Decapoda)	–	–	*	–	–	–	*	–	–	–	–	–	–	–
Octopuses (Octopoda)	–	–	–	–	–	–	*	–	–	–	*	–	–	*
Chitons (Polyplacophora)	–	–	*	–	*	–	–	–	*	–	*	–	–	–
Total	1	1	5	1	7	10	7	8	7	8	8	7	3	9

Echinoderms														
Unidentified	9	–	11	–	12	*	11	1	10	*	9	–	–	*
Starfish (Asteriidae)	–	–	1	–	–	*	*	*	–	*	*	–	–	*
Brittle stars (Ophiurae)	8	–	3	–	4	–	6	*	7	–	6	*	*	*
Sea urchins (Cidaroidea)	–	–	1	–	3	–	1	–	6	–	2	*	–	*
Basket stars (Euryalae)	–	–	1	–	–	–	*	–	*	–	*	–	–	–
Sand dollars (Scutellidae)	2	–	1	–	4	–	1	–	3	–	1	*	*	–
Sea cucumbers (Holothuroidea)	–	–	–	–	*	1	–	–	–	–	1	–	*	*
Psolidae	–	–	–	–	*	–	–	–	–	–	–	–	–	–
Total	19	–	17	–	23	1	20	2	27	*	20	1	1	*
Others														
Fish eggs	–	–	*	–	*	2	1	1	*	*	3	1	*	*
Ascidians	–	–	*	–	–	–	1	–	*	*	3	–	–	–
Ctenophores	–	–	*	–	2	–	1	–	*	*	*	*	*	*
Coelenterates	–	–	*	–	*	1	*	–	1	*	1	*	*	*
Poriferans	–	–	–	–	–	–	*	–	*	*	*	*	*	*
Arrow worms (Chaetognatha)	–	–	*	*	–	–	*	–	–	–	–	–	–	–
Total	–	–	*	*	2	3	3	1	2	1	7	1	*	*
Unidentified														
Animals	12	7	12	6	7	1	4	1	2	1	2	2	*	*
Mucus	9	3	8	1	7	2	5	2	5	1	4	1	*	2
Stones, mud, etc.	*	–	–	–	*	–	–	–	*	*	*	*	*	*
Total	21	10	20	7	14	3	9	3	7	2	6	3	1	2
Grand total	100	99	100	99	99	99	98	99	98	100	98	99	99	98

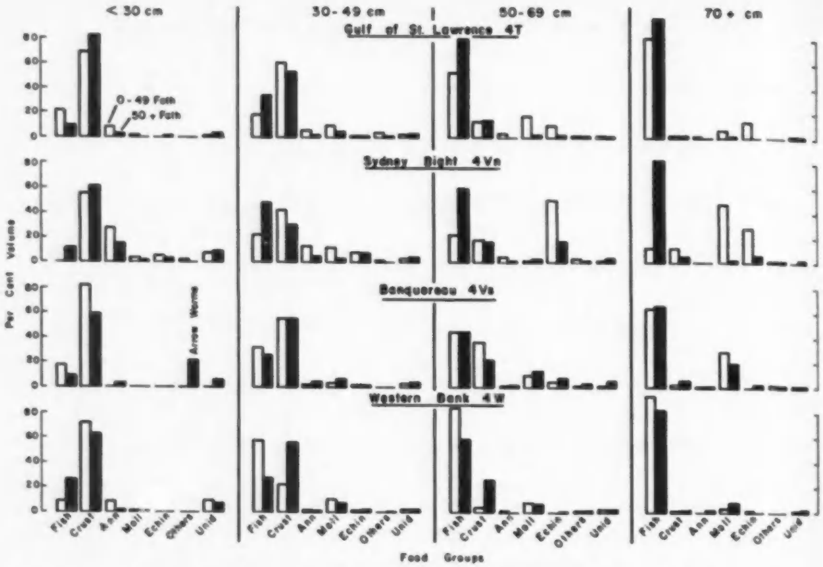

FIG. 2. Percentage volumes of stomach contents of cod by size of fish, study area, and depth, in the January–April period, 1958–63.

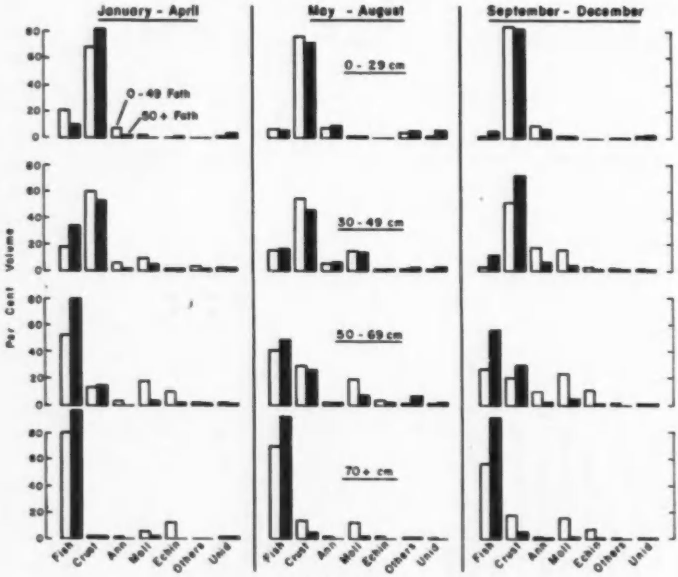

FIG. 3. Percentage volumes of stomach contents of cod from the Gulf of St. Lawrence (Division 4T) by size of fish, depth, and season.

year. Percentage volumes of fish items decreased slightly in shoaler depths as the year progressed, and those of molluscs and echinoderms tended to increase.

In the Sydney Bight area (Fig. 4) during the period May–August, cod up to 49 cm long fed to a great extent on larval ascidians in shoal water and on larval ascidians, ctenophores, and *Sagitta* in deep water. In contrast, few of these items were eaten in January–April. The diet of the large cod (50+ cm) differed between seasons, mainly in the relative quantities of fish, molluscs, and echinoderms.

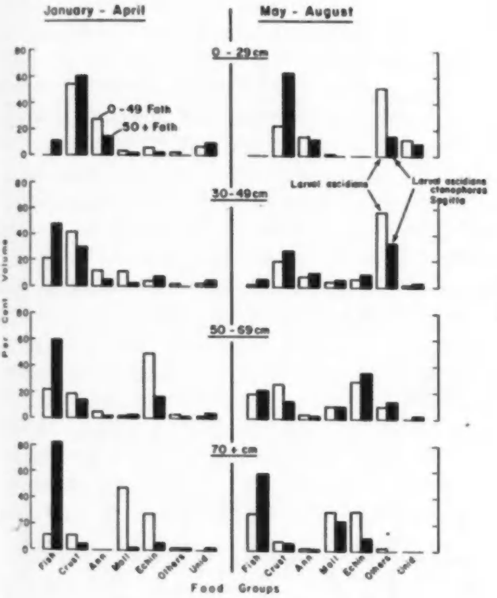

FIG. 4. Percentage volumes of stomach contents of cod from Sydney Bight (Division 4Vn) by size of fish, depth, and season.

On Western Bank (Fig. 5) there were some differences in relative volumes of fish and crustaceans between the January–April and May–August periods. Less fish and more crustaceans were evident in all but the 70+-cm fish in the latter period. Another major difference was that the percentage volume of molluscs in 50+-cm cod from shoal water was higher in May–August than in January–April.

IMPORTANCE OF SPECIFIC ITEMS

Many of the smaller fishes common to the study areas and captured by the lined otter trawl appeared in the cod stomachs, but only a few were found with sufficient frequency to be considered important food items (Fig. 6).

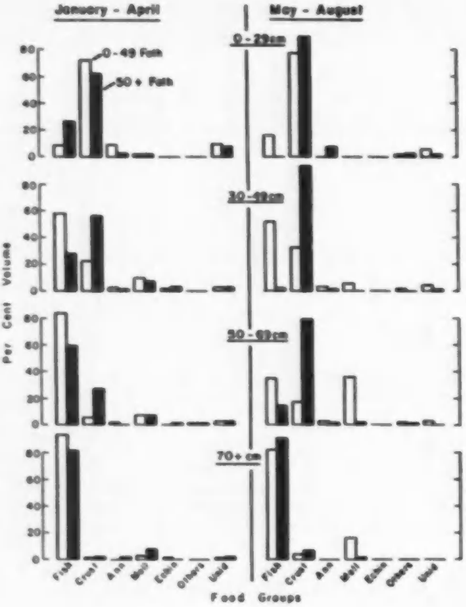

FIG. 5. Percentage volumes of stomach contents of cod from Western Bank (Division 4W) by size of fish, depth, and season.

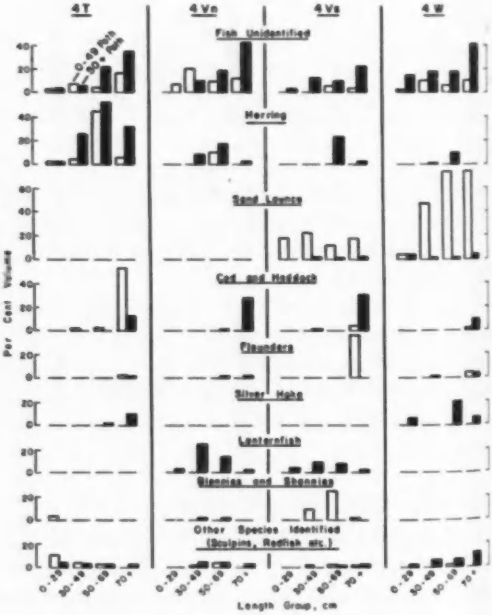

FIG. 6. Percentage volumes of fish found in cod stomachs by area, depth, and size of cod, seasons combined.

Herring was the most important fish item in the Gulf of St. Lawrence (4T) as was sand launce in shoal water in the Western Bank region (4W). Herring appeared less frequently in 4Vn than in 4T and was also relatively unimportant in 4Vs and 4W.

Sand launce occurred in the diet of cod on Banquereau (4Vs) but were less important than on Western Bank (4W). They were much more common in the stomachs of fish from shoal than from deep water.

Smaller haddock and flounders (Pleuronectidae) were found mainly in large cod (70+ cm) and may have been captured as an alternative to herring and sand launce when the latter two species were not available. Lantern fish (Myctophidae) occurred frequently in cod stomachs in Divisions 4Vn and 4Vs deep water (over 100 fath (ca. 183 m)), where these species are known to be fairly abundant. Very few tows were made in over 100 fath of water in Divisions 4W and 4T, and this may account for the fact that no lantern fishes were found in cod stomachs in these areas. Species occurring less frequently or in small quantities were: silver hake (*Merluccius bilinearis*), blennies and shannies (Stichaeidae), sculpins (Cottidae), and redfish (*Sebastes* spp.).

Fish items tend to digest rapidly and some were listed as unidentifiable to species. Likely much of this quantity was made up of the same species as the identifiable species for the particular area, depth, and size-group.

As mentioned earlier, crustaceans were the most important items in the diet of small cod (Fig. 2). The proportion of crustaceans decreased with increasing size of cod to negligible amounts in the 70+-cm group. The most important items within the class (Fig. 7) were euphausids, pandalids, amphipods, mysids, and decapods, particularly crabs (Brachyura) including hermit

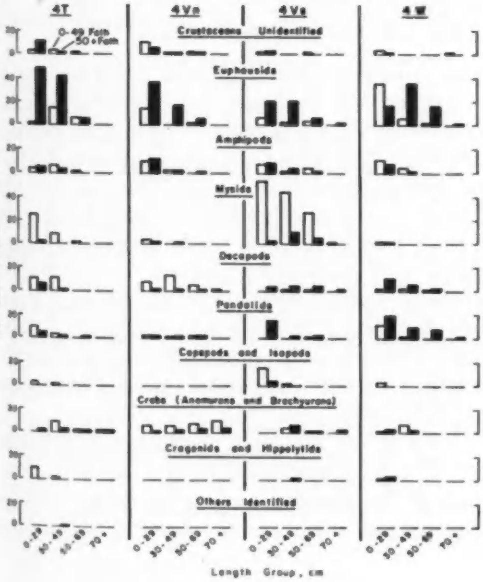

FIG. 7. Percentage volumes of crustaceans found in cod stomachs by area, depth, and size of cod, seasons combined.

crabs (Paguridae). Mysids were particularly important in the diet of cod in Division 4Vs shoal water. Euphausids and pandalids were more prominent among crustaceans in the cod's diet in deeper water, euphausids in all four areas, and pandalids in divisions 4Vs and 4W.

Several factors appeared to influence the diet of cod, among them the size of food item, the availability or abundance, and the ease of capture. It has been demonstrated by experiments on bait selection (Martin and McCracken, MS, 1954) that cod show a preference for or select certain types of bait (i.e. food). This study showed that fish were selected over other available types of natural food by the larger cod, and also at times by the smaller cod where the fish prey was small, such as sand launce eaten in Division 4W and lantern fish in divisions 4Vn and 4Vs. Cod caught in shoal water contained prey normally present in shoal water, i.e. sand launce in divisions 4Vs and 4W, small cod in Division 4T, small flounders in Division 4Vs. Similarly, in deep water, silver hake were found in cod stomachs in divisions 4T and 4W, and lantern fish in divisions 4Vn and 4Vs (Fig. 6).

Certain items or species within the class Crustacea also tended to be utilized to a greater extent in certain areas and depths (Fig. 7), presumably where they are the most abundant. These included euphausids in deep water in all areas and in shallow water in divisions 4Vn and 4W; mysids in shallow water in 4Vs; pandalids in deep water in 4W; and crabs (Brachyurans) in 4Vn.

Large cod tended to supplement their fish diet by feeding on bivalve molluscs and brittle stars (Ophiurae) (Fig. 2). This was particularly evident for 70+-cm cod in Division 4Vn and to a lesser extent in 4Vs and 4T.

FOOD SELECTION OF COD AND HADDOCK

Western and Emerald Bank (Division 4W) data were examined to determine if overlap existed between cod and haddock in use of available food in this area where both species were relatively abundant. The data available were from *A. T. Cameron* cruises for 1959–62 in the Western and Emerald Bank areas, January–April, and included observations on 2741 cod and 4710 haddock stomachs containing food. Size-groups and depth ranges were the same as used in the cod food study except that very few haddock exceeded 69 cm long and these were included with the 50–69-cm group.

The average volume of stomach contents (Fig. 8) increased with length of fish in both depth zones, and the rate of increase of volume was greater than the rate of increase in length. Cod stomachs contained from two to three times as much food as haddock stomachs, with the ratio increasing with larger sizes.

A comparison of stomach contents of cod and haddock by individual items (Table 2) and food groups (Fig. 9) showed that more than 80% of the food of both cod and haddock could be classified under the group headings: fish, crustaceans, annelids, molluscs, and echinoderms. Less than 5% were

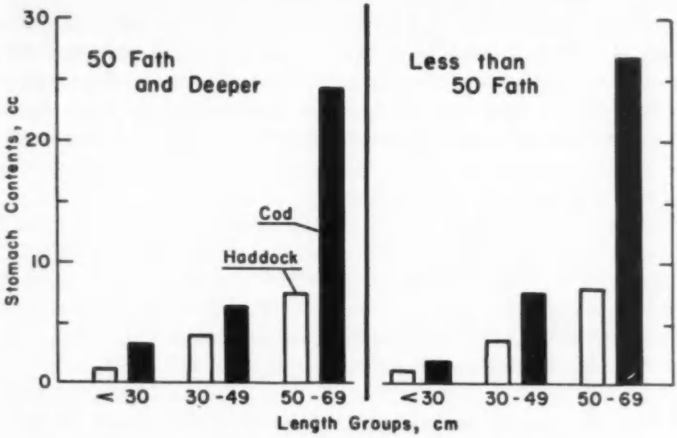

FIG. 8. Average volumes of stomach contents of cod and haddock from ICNAF Division 4W by size of fish and depth, January–April, 1959–62.

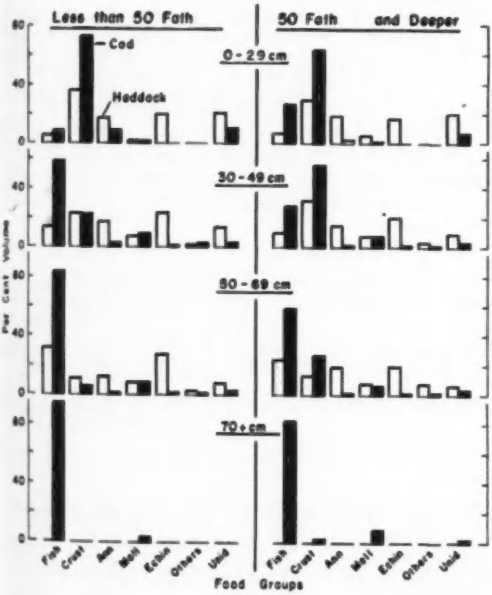

FIG. 9. Percentage volumes of various food groups eaten by cod and haddock from ICNAF Division 4W by size of fish and depth zone, January–April, 1959–62.

in the phyla Ctenophora, Porifera, and Chaetognatha. Approximately 10% was unidentified or listed as mucus.

The most important items in the diet of small cod and haddock were crustaceans. Fish became increasingly important as food of larger fish but was taken more often by cod than by haddock. Cod, in general, fed more on pelagic crustaceans and fish than did haddock, whereas haddock fed more on bottom animals such as annelid worms and echinoderms than did cod.

Cod and haddock selected the same types or items of food but the percentage volumes of individual items were quite different (Fig. 10). Of the fish items, sand launce was the most important item for both species at depths of less than 50 fath. For cod, it was the most important of all food items at this depth range. Herring, silver hake, and argentine were the main fish items for both species at depths greater than 50 fath. Our survey tows have shown that sand launce normally inhabit the shoaler depths whereas silver hake, herring, and argentines (*Argentina silus*) are normally found in deep water at this season of the year (January–April).

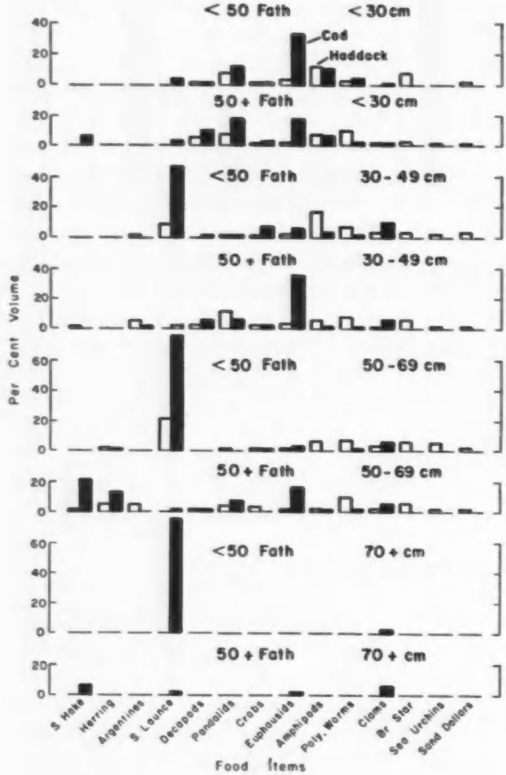

FIG. 10. Percentage volumes of various food items eaten by cod and haddock on Western and Emerald banks (Division 4W) by depth and size of fish, January–April, 1959–62.

All of the crustacean items listed, of any dietary importance, were utilized by both species (Table 2). Many more euphausids were taken by cod than by haddock. Other important items for both species were decapods, including crabs, and pandalids. Polychaete worms, brittle stars, sea urchins, and sand dollars (Scutellidae) were the main items that were common in the diet of haddock but of little importance in the diet of cod, indicating that haddock tend to feed more on bottom fauna than do cod. In fact, it was here that the greatest contrast appeared in the food items of the two fishes. The benthic forms of the annelid worms, molluscs, and echinoderms found as food items made up more than 40% of the total volume for haddock and less than 10% of the total for cod. Brunel (1963) found similar feeding preferences in cod off Grande-Rivière. There, they tended to feed on pelagic prey, when the latter were abundant, with a resulting decrease in the quantity of benthic prey found in the stomachs. Templeman (1964) also observed that active prey, such as capelin (*Mallotus villosus*), lancet fish (*Paralepis coregonoides borealis*), and lantern fish appeared to attract cod into particular schooling and feeding patterns.

It appears that when there is an abundance of pelagic prey, particularly fish, as well as benthic food in an area where both cod an haddock are abundant, there is less likelihood of competition between the two species for the same food. Cod would tend to feed on the pelagic, more active prey, whereas haddock would concentrate mainly on the slower moving or sedentary organisms.

ACKNOWLEDGMENTS

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REFERENCES

Brunel, Pierre. 1963. Variations journalières et saisonnières de l'alimentation de la morue au large de Grande-Rivière. Rappt. Ann. 1962, Station de biologie marine, Grande-Rivière, Québec. p. 101–113.

Corbeil, H.-E. 1954. Analyse du contenu stomacal de la morue (*Gadus callarias*). Contrib. Dépt. Pêcheries, Québec 50: 48–59.

Homans, R. E. S., and A. W. H. Needler. 1944. Food of the haddock. Proc. N.S. Inst. Sci. 21: 15–49.

Jean, Yves. 1964. Seasonal distribution of cod (*Gadus morhua* L.) along the Canadian Atlantic coast in relation to water temperature. J. Fish. Res. Bd. Canada 21: 429–460.

Martin, W. R., and F. D. McCracken. MS, 1954. Relative efficacy of baits for groundfish. Fish. Res. Bd. Canada, Atlantic Progr. Rept. 58: 17–20.

Powles, P. M. 1958. Studies of reproduction and feeding of Atlantic cod (*Gadus callarias* L.) in the southwestern Gulf of St. Lawrence. J. Fish. Res. Bd. Canada 15: 1383–1402.

Templeman, W. 1964. Some instances of cod and haddock behaviour and concentrations in the Newfoundland and Labrador areas in relation to food. Intern. Comm. Northwest Atlantic Fish., Spec. Publ. 6: 449–461.

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Age, Growth, and Maturity of the Longnose Sucker *Catostomus catostomus*, of Western Lake Superior[1]

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BAILEY, MERRYLL M. 1969. Age, growth, and maturity of the longnose sucker *Catostomus catostomus*, of western Lake Superior. J. Fish. Res. Bd. Canada 26: 1289–1299.

Studies of age, growth, and maturity were based on 1760 fish collected in western Lake Superior in 1964–65. The body:scale relation was curvilinear and the curve had an intercept of 1.65 inches on the length axis. The weight increased as the 2.85 power of the length. Some fish formed an annulus before May 18 in 1965; all had completed annuli by late September. Longnose suckers grew 3.6 inches the 1st year, reached 12 inches in the 6th year, and 18 inches in the 11th year. Fish from Pikes Bay grew faster than those from Gull Island Shoal. Over 6 years were required for weight to reach 1 lb and nearly 10 years to reach 2 lb. Minimum length at maturity was 10.5 inches for males and 11.5 inches for females. The youngest mature male belonged to age-group IV and the youngest mature female to age-group V. All males were mature at 14.5–14.9 inches (age-group VIII) and all females at 15.0–15.4 (age-group IX).

Finclipped longnose suckers returned to spawn in the Brule River in successive years. One fish returned to spawn in 4 successive years. Many of the fish were not recaptured until 2 or 3 years after marking. The time of the Brule River spawning migration depended more on water temperature than on length of day. The average water temperature during the peak of the spawning runs of 1958–64 was 55.4 F. Larval suckers apparently spend little time in the Brule River and adjacent streams and drift downstream to the lake soon after hatching. The number of eggs in the ovaries of eight suckers ranged from 14 to 35 thousand and averaged 24 thousand for fish 13.9–17.7 inches long.

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INTRODUCTION

THE LONGNOSE SUCKER (*Catostomus catostomus*) ranges from Alaska and northern Canada, south to the northern Rocky Mountains and to the northern margin of the Mississippi River system in Minnesota, and eastward through the St. Lawrence River drainage and northeastern New England. The fish are found in all of the Great Lakes but are resident in inland lakes only in the Lake Superior drainage (Hubbs and Lagler, 1958).

The longnose sucker is easily distinguished from the nearly 100 other species of suckers in North America by the small size of its scales (more than 85 in the lateral line) and, as its name implies, by its long protruding snout. Suckers, including the white sucker (*Catostomus commersoni*), the red horse

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(*Moxostoma* spp.) and *C. catostomus* are commonly referred to as "mullet" by commercial fishermen in the Great Lakes. Although the longnose sucker is extremely abundant in some inshore areas of Lake Superior, its commercial value is small.

The annual commercial catch of mullet in the U.S. waters of Lake Superior has never exceeded 0.5 million lb and averaged only 145 thousand in 1929–60 (Baldwin and Saalfeld, 1962). The contribution of the longnose sucker to the total Great Lakes production of mullet is small.

Growth of *C. catostomus* has been studied in Yellowstone Lake (Brown and Graham, 1954), the Missouri River (Kathrein, 1951), Pyramid Lake, Alberta (Rawson and Elsey, 1950), Great Slave Lake (Harris, 1962), and Sixteenmile Lake, British Columbia (Green et al., 1966). The present report is the first for the Great Lakes.

MATERIALS AND METHODS

Analyses of age, growth, and maturity were based on 1760 longnose suckers collected in experimental gillnets and trawls fished by the Bureau of Commercial Fisheries research vessels *Siscowet* and *Kaho* in western Lake Superior, 1964–65 (Table 1). Some fish were also taken from fish traps at the electrical sea lamprey barrier on the Brule River (Fig. 1).

The experimental nylon gillnets were 6 ft deep, 250–600 ft long, and had mesh sizes of 0.75–6 inches, stretched measure. The nets were fished on the bottom either singly or in gangs of 2–12 nets.

The semiballoon bottom trawls fished by the *Siscowet* were 40 ft long with a 38-ft bottom line, 2-inch mesh (stretched measure) body, and 0.5-inch mesh cod end. The trawls fished by

TABLE 1. Numbers of longnose suckers studied from western Lake Superior, and gears and locations of capture, 1964–65.

Date of collections	Gear	Location	No. of fish
1964			
Mar. 12	Gillnets	Apostle Islands	3
May 2	Weir trap	Brule River	178
May 13–20	Gillnets	Apostle Islands	113
June 23	"	Apostle Islands	116
Aug. 11	"	Bark Bay	136
Sept. 23–Oct. 7	"	Apostle Islands	153
Oct. 15–27	"	" "	256
Nov. 16	"	" "	2
1965			
May 18	"	" "	36
June 15	"	" "	64
June 16	Otter trawl	Duluth	308
July 20	Gillnets	Apostle Islands	103
Aug. 31	"	" "	10
Sept. 22–28	"	" "	65
Oct. 5	Otter trawl	Duluth	217
Total:			1760

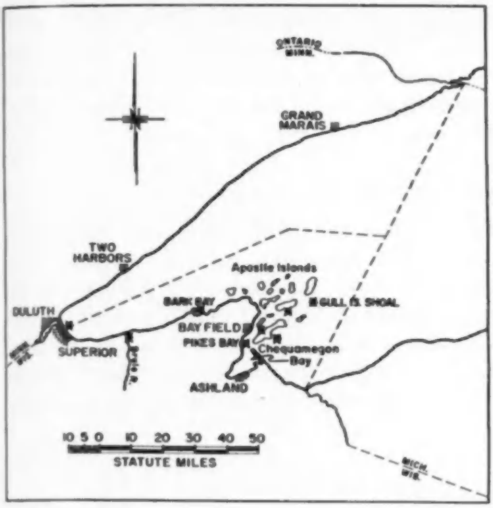

FIG. 1. Western Lake Superior. Dots represent sampling stations.

the *Kaho* were Gulf of Mexico shrimp nets with a 52-ft headrope and 1-inch mesh cod end liner. Trawls were dragged along the bottom in 3–12 fath for either 15 or 30 min.

The fish traps were similar to those described by McLain et al. (1965). The sea lamprey barrier and traps on the Brule River were about 1 mile upstream from Lake Superior.

Field techniques and analyses of data were similar to those described by Bailey (1964).

Sex was easily distinguished for mature suckers but was questionable for immature fish. Sex data for fish of age-groups 0–III, therefore, are excluded.

The body:scale relation was based on the average diameters of three to five scales from each of 1391 fish. A graphic plot of the average scale diameters (S) and mean total body lengths (L) indicated that the data were curvilinear. Logarithmic plots of $L = cS^n$ and $L-a = cS^n$ (a values of 1.9 and 2.0, c and n constant) were not linear; a curve was accordingly fitted by inspection. The intercept on the length axis was 1.65 inches. A nomograph was constructed on the basis of the empirical curve. Lengths at each annulus were read from a nomograph based on the fitted line.

A general length–weight relation was determined from data from 1412 longnose suckers 5.8–19.8 inches in total length, and had the equation:

$$\log W = -1.19426 + 2.8499 \log L,$$

where W = weight in lb, and L = total length in inches. Suckers from Gull Island Shoal tended to be heavier for a given length than those from other locations. I believe, however, that the best description of a general length–weight relation is based on all available fish.

Ages corresponding to the numbers of annuli are designated by Roman numerals. All the fish were considered to have passed into the next age-group on January 1.

Scales from a small number of fish (1.3%) were discarded as unreadable.

TIME OF ANNULUS FORMATION

The period of annulus formation for 791 suckers from Apostle Island and Bark Bay extended over 3–4 months and varied with age of fish and year of collection. The fish formed annuli earlier in 1965 than in 1964. The percentage of fish of all ages with new growth was 16.7% on May 18, 1965, but was nil for fish taken on May 13–16, 1964. The percentage of fish with new growth increased progressively through August, and all fish had started to grow by late September in both years. Little evidence was found that the younger suckers form annuli earlier than the older ones.

LENGTH DISTRIBUTIONS OF THE AGE-GROUPS

For 1349 longnose suckers from Duluth, the Brule River, and the Apostle Islands the range in length of the various age-groups varied from 3.0 inches for fish of age-groups I, II, and XI to 9.0 inches for age-V fish (Table 2). The distribution of age-III fish completely overlapped that of the age-II fish and ranged 5.0 inches. Length intervals from 13.0 to 18.9 inches included four to six age-groups. The extensive range in length and overlap of the individual age-groups was undoubtedly influenced by the combination of samples of fish that grew at different rates.

GROWTH IN LENGTH AND WEIGHT

Longnose suckers from western Lake Superior grew 3.6 inches in their 1st year of life, reached 12 inches in their 6th year, and 18 inches in their 11th year (Table 3). Between the 2nd and 6th year of life annual growth increments were nearly identical (1.8–1.9 inches), but the increment declined in the 7th year and was smallest (0.8 inch) in the 10th year. The calculated lengths did not differ by sex or date of capture but did vary by area.

Growth of suckers from 1.5- to 3-inch mesh gillnets differed considerably between Pikes Bay and Gull Island Shoal (Fig. 1). Fish from Pikes Bay were 0.4 inch longer at the end of the 1st year of life, and were 1.8 inches longer by the end of the 5th year. The trend reversed in the 6th through 8th years and by the end of the 8th year fish from Gull Island Shoal were only 0.6 inch shorter than those from Pikes Bay. Growth differences between the two areas conceivably could be caused by the presence of genetically distinct subpopulations, but environmental differences offer a more likely explanation. Pikes Bay is somewhat eutrophic; it is relatively warm and shallow and has extensive beds of aquatic vegetation. Conversely, the waters surrounding Gull Island Shoal are typically oligotrophic.

Brown and Graham (1954) calculated average total lengths for 525 *C. catostomus* from Yellowstone Lake, Wyoming, and Kathrein (1951) gave growth data for 87 fish from the Missouri River in Montana. Back calculations in both studies were apparently by direct proportion. To permit valid comparisons among fish from all three areas, back calculations for fish from Lake Superior were recalculated by direct proportion (Table 4).

Longnose suckers from western Lake Superior were longer after the 1st growing season than fish from the other two areas. Second-year growth in all three areas was similar (2.5–2.8 inches), but fish from Yellowstone Lake grew faster during the 3rd and 4th years and by the end of the 4th year were 1.1

TABLE 2. Length distributions of age-groups of longnose suckers from western Lake Superior (based on actual lengths of fish that had not started current season's growth or on calculated length at last annulus).

Length interval (*inches*)	No. of fish	Age-groups										
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI
3.0–3.9	28	28	–	–	–	–	–	–	–	–	–	–
4.0–4.9	23	23	–	–	–	–	–	–	–	–	–	–
5.0–5.9	43	3	31	9	–	–	–	–	–	–	–	–
6.0–6.9	30	–	18	12	–	–	–	–	–	–	–	–
7.0–7.9	53	–	3	31	18	1	–	–	–	–	–	–
8.0–8.9	110	–	–	19	82	9	–	–	–	–	–	–
9.0–9.9	131	–	–	3	69	57	2	–	–	–	–	–
10.0–10.9	171	–	–	–	25	137	9	–	–	–	–	–
11.0–11.9	81	–	–	–	1	54	22	4	–	–	–	–
12.0–12.9	96	–	–	–	–	25	52	19	–	–	–	–
13.0–13.9	183	–	–	–	–	15	94	63	11	–	–	–
14.0–14.9	173	–	–	–	–	3	48	91	29	1	1	–
15.0–15.9	121	–	–	–	–	2	26	45	38	9	1	–
16.0–16.9	66	–	–	–	–	–	6	21	30	7	2	–
17.0–17.9	26	–	–	–	–	–	–	2	13	4	5	2
18.0–18.9	12	–	–	–	–	–	–	2	2	5	3	–
19.0–19.9	2	–	–	–	–	–	–	–	–	1	–	1
Total:	1349	54	52	74	195	303	259	247	123	27	12	3
Avg length (*inches*):		4.0	6.0	7.4	9.0	10.8	13.4	14.5	15.6	16.7	17.2	18.2
Range (*inches*):		3.0	3.0	5.0	5.0	9.0	8.0	8.0	6.0	6.0	5.0	3.0

TABLE 3. Calculated total lengths for each year of life of longnose suckers from western Lake Superior.

		Length (*inches*) at end of year										
Age-group	No. of fish	1	2	3	4	5	6	7	8	9	10	11
I	54	4.1	–	–	–	–	–	–	–	–	–	–
II	52	4.1	6.0	–	–	–	–	–	–	–	–	–
III	74	3.8	5.7	7.4	–	–	–	–	–	–	–	–
IV	195	3.8	5.5	7.2	9.0	–	–	–	–	–	–	–
V	303	3.6	5.4	7.3	9.1	10.7	–	–	–	–	–	–
VI	259	3.6	5.6	7.7	9.8	11.7	13.4	–	–	–	–	–
VII	247	3.4	5.4	7.3	9.2	11.1	12.8	14.4	–	–	–	–
VIII	123	3.3	5.1	6.9	8.8	10.6	12.4	13.9	15.5	–	–	–
IX	27	3.0	4.6	6.1	7.8	9.6	11.4	13.2	14.8	16.7	–	–
X	12	3.0	4.7	6.2	7.6	9.4	11.3	12.9	14.3	15.7	17.2	–
XI	3	2.8	4.3	5.6	7.2	8.6	9.8	11.3	13.4	15.0	16.5	18.3
Total or avg	1349	3.6	5.4	7.3	9.2	11.0	12.9	14.1	15.3	16.3	17.1	18.3
Increment of avg	–	3.6	1.8	1.9	1.9	1.8	1.9	1.2	1.2	1.0	0.8	1.2

TABLE 4. Calculated total lengths for each year of life of longnose suckers from western Lake Superior, Yellowstone Lake, and the Missouri River[a] (all calculations by direct proportion).

	Year of life							
Item	1	2	3	4	5	6	7	8
Western Lake Superior								
Avg calculated length	3.2	5.9	8.4	10.6	12.7	14.6	15.7	16.7
Increment of avg	3.2	2.7	2.5	2.2	2.1	1.9	1.1	1.0
Yellowstone Lake								
Avg calculated length	2.0	4.8	8.5	11.7	13.6	15.8	17.4	18.1
Increment of avg	2.0	2.8	3.7	3.2	1.9	2.2	1.6	0.7
Missouri River								
Avg calculated length	3.0	5.5	8.1	10.4	12.3	13.7	14.7	15.5
Increment of avg	3.0	2.5	2.6	2.3	1.9	1.4	1.0	0.8

[a] Data for Yellowstone Lake are from Brown and Graham (1954), and those for the Missouri River are from Kathrein (1951). The data included no fish beyond the 8th year of life.

inches longer than fish from Lake Superior and 1.3 inches longer than those from the Missouri River. Growth was again similar during the 5th year (1.9–2.1 inches), but thereafter fish from the Missouri River grew slower and fish from Yellowstone Lake grew faster than those from western Lake Superior.

Green et al. (1966) estimated (from length–frequency distributions) an average annual increment of 15–20 mm (0.6–0.8 inch) for mature suckers in Sixteenmile Lake, British Columbia, and Harris (1962) estimated yearly growth to be 20–25 mm (0.8–1.0 inch) in Great Slave Lake. These estimates are slightly less than the average annual growth increments for mature fish in the three areas compared in Table 5.

Weight increments for fish from western Lake Superior increased progressively from 0.02 lb in the 1st year of life to 0.35 lb in the 6th. In the 7th through 10th years, increments were 0.27–0.31 lb. Over 6 years were required for suckers to reach 1 lb and nearly 10 years to reach 2 lb.

SEX RATIO AND MATURITY

Sex composition of age-groups IV–X was determined from 895 longnose suckers taken in 1964–65. The percentage of males exceeded that of females in age-groups IV–VI (55.6–59.7% males); the sexes were nearly equally represented for age-groups VII and VIII; and females exceeded males in age-groups IX and X (75.0–90.0% females).

The shortest mature male was 10.5 inches and the shortest mature female was 11.5 inches. Over 20% of the males were mature at 11.5–11.9 inches whereas only 3.6% of the females were mature at the same length. All the males were mature at 14.5–14.9 inches and all the females at 15.0–15.4 inches.

TABLE 5. Daily catches of longnose suckers at sea lamprey barrier in Brule River, and daily water temperatures (F) averaged for 5-day periods, April–June, 1958–64. (The highest catches and corresponding water temperatures are in italics.)

	Year													
	1958		1959		1960		1961		1962		1963		1964	
Date	No.	Temp	No.	Temp	No.	Temp	No.	Temp	No.	Temp	No.	Temp	No.	Temp
April														
12–16	123	52.4	–	–	–	–	–	–	–	–	–	–	–	–
17–21	*482*	*55.4*	69	45.0	11	47.6	–	–	–	–	16	46.4	–	–
22–26	17	48.4	241	50.4	44	44.0	–	–	212	50.0	44	48.8	10	48.0
27–May 1	2	46.8	364	51.4	15	46.2	40	43.7	92	45.6	72	50.2	48	45.2
May														
2–6	43	54.2	*443*	*55.2*	137	46.6	181	47.6	214	56.2	39	51.0	*87*	*51.6*
7–11	95	59.0	426	55.0	350	48.0	227	47.4	16	46.8	*202*	*55.2*	7	56.7
12–16	10	64.8	113	51.6	*653*	*57.6*	*1040*	*55.0*	98	53.8	2	48.0	2	54.2
17–21	31	60.8	245	54.4	9	59.0	808	52.8	88	64.3	125	51.0	3	58.0
22–26	–	–	203	56.0	403	60.2	308	57.0	*427*	*58.0*	60	55.7	7	63.0
27–31	–	–	74	57.4	243	57.2	198	59.6	355	58.8	29	56.0	–	–
June														
1–5	–	–	52	63.8	258	62.2	18	60.0	76	54.8	–	–	–	–
6–10	–	–	–	–	36	60.4	–	–	3	58.3	–	–	–	–
11–15	–	–	–	–	13	64.8	–	–	–	–	–	–	–	–

The youngest mature males belonged to age-group IV, and all males were mature at age VIII. Females first matured at age V and all were mature at age IX.

BRULE RIVER SPAWNING POPULATION

An electrical barrier for experimental control of the sea lamprey (*Petromyzon marinus*) was first operated on the Brule River in the spring of 1957. Traps installed at the barrier captured sea lampreys and large numbers of longnose suckers on their upstream spawning migrations. To study the homing characteristics of the suckers, 23,506 fish were marked by removal of a pectoral or pelvic fin during the spawning seasons of 1958–61. Fish recaptured in 1959–61 were marked again by removing an additional fin. All marked fish were released downstream from the traps.

In 1962–64 records were kept on the number of fish recaptured but the fish were not marked again. The returns for 1962–64, therefore, may be too high because some fish may have been counted more than once.

The returns from finclipped longnose suckers show that some fish returned to spawn in the Brule River in successive years. First-year returns ranged from 17.6% in 1960 to 6.8% in 1961. The percentage of fish recaptured in 2 successive years ranged from 2.5 to 0.6%. One fish marked in 1958 was recaptured in 4 sucessive years.

Many of the finclipped suckers were not recaptured until 2 or 3 years after marking. The percentage of fish recaptured for the first time 2 years after marking ranged from 10.5 to 4.5%. Over 3% of the fish marked in 1958 were recaptured for the first time in 1961 (3 years after marking).

It is not known whether some longnose suckers spawn in alternate years, or in different streams, or whether some of the fish spawned each year in the Brule River but did not enter the traps.

Green et al. (1966) reported that 20–25% of the longnose suckers they marked in a tributary stream of Sixteenmile Lake, British Columbia, returned to spawn the following year. Second-year returns ranged from 10 to 30%; 0.8% of the fish returned 6 years after marking. Olson and Scidmore (1963) showed definite evidence of homing for white suckers in Many Point Lake in northern Minnesota. Of 5226 fish marked at one inlet, 9.7% returned to spawn the following spring. The recaptured fish were marked a second time and a remarkably high 74.2% (377/508) spawned at the same location for the 3rd consecutive year. Dence (1948) found that dwarf suckers, *Catostomus commersonii utawana*, in Wolf Lake, New York, usually returned each year to spawn in the same stream. The percentages of fish recaptured the year after marking ranged from 14.5 to 44.9 during his 5-year study.

A comparison between average daily catches and water temperatures in the Brule River (Table 5) suggests that spawning depended on water temperature. During the 5-day intervals of highest catch in the various years, mean water temperatures ranged from 51.6 F in 1964 to 58.0 F in 1962 and averaged 55.4 F for the 7-year period. The dates of peak migration, however, varied by 36 days — from April 19 (1958) to May 25 (1962). The spawning migration generally intensified as the river water temperatures approached 55 F and then declined as the temperatures dropped. A second increase in the catches usually followed a second rise in water temperature, but the catches fell off sharply when water temperatures exceeded 60 F.

A relation between water temperature and spawning has been observed by other workers. Brown and Graham (1954) found peak spawning in a tributary stream to Yellowstone Lake between July 5 and 9 when water temperatures were 54–59 F. Harris (1962) stated that in a tributary to Great Slave Lake most longnose suckers spawned between June 6 and 18, also when water temperatures were 54–59 F. In Fry Creek, British Columbia, Green et al. (1966) found that the majority of longnose suckers spawned May 1–20 when water temperatures were 50–52 F. Rawson and Elsey (1950) reported that the suckers began to spawn about June 10 in Pyramid Lake, Alberta, when water temperatures were 51.8–57.2 F.

Data on the sex and age compositions and the average lengths of 178 spawning-run suckers were collected on May 2, 1964, at the Brule River barrier. Males outnumbered females 91 to 79 and the mean length of males (15.2 inches) was 1.4 inches less than that of females (16.6 inches). The average age of males (7.2 years) was also less than that of females (8.0 years). Age-groups VI–VIII made up 91.2% of the males and age-groups VII–IX made up 75.9% of the females.

Although seining in tributary streams of Yellowstone Lake (Brown and Graham, 1954) and in streams in Canada yielded age-0 longnose suckers in July and August, attempts to locate young-of-the-year in the Brule River and adjacent streams in 1964–65 were unsuccessful. Seining in the streams and trawling near the mouths of the streams yielded young-of-the-year white suckers but not longnose suckers. The only age-0 longnose suckers taken in the present study were captured in trawls fished about 0.5 mile off Park Point at Duluth, Minnesota, on October 5, 1965. Larval suckers apparently spend little time in the Brule River and adjacent streams, but drift to the lake soon after hatching. The Bureau of Commercial Fisheries sea lamprey stream treatment and survey crews have never observed an age-0 longnose sucker in a stream tributary to Lake Superior.

FECUNDITY

Estimates of the number of eggs in the ovaries of eight longnose suckers taken in the spring of 1964 were determined volumetrically. The average number of eggs was 26 thousand and ranged from nearly 14 thousand for the shortest fish (13.9 inches) to over 35 thousand for a 17.7-inch fish. The average number of eggs per ounce of body weight increased irregularly from 714 for a 13.9-inch fish to 1100 for a 17.7-inch fish. The mean diameter of 12 eggs from one gravid female was 2.2 mm.

Egg production for eight suckers from Great Slave Lake (Harris, 1962) ranged from 17 to 60 thousand and averaged 35 thousand for fish 450–568 mm

(17.7–22.4 inches), fork length. These estimates from Great Slave Lake appear to be lower for a given length than for fish from Lake Superior, but different methods of fish measurement make comparisons difficult. Egg diameters of fish from Great Slave Lake were 3.0 mm compared with 2.2 mm for Lake Superior fish. The difference in egg size may account for the apparent difference in the number of eggs in fish of similar lengths.

ACKNOWLEDGMENTS

I thank Harry Moore of the Bureau of Commercial Fisheries Biological Station, Marquette, Michigan, who provided the data on the Brule River spawning migrations.

REFERENCES

Bailey, M. M. 1964. Age, growth, maturity, and sex composition of American smelt, *Osmerus mordax* (Mitchill), of western Lake Superior. Trans. Am. Fish. Soc. 93: 382–395.

Baldwin, N. S., and R. W. Saalfeld. 1962. Commercial fish production in the Great Lakes, 1867–1960. Great Lakes Fish. Comm. Tech. Rept. 3. 166 p.

Brown, C. J. D., and R. J. Graham. 1954. Observations on the longnose sucker in Yellowstone Lake. Trans. Am. Fish. Soc. 83: 38–46.

Dence, W. A. 1948. Life history, ecology and habits of the dwarf sucker, *Catostomus commersonii utawana* Mather, at Huntington Wildlife Station. Roosevelt Wildlife Bull. 8: 81–150.

Green, G. S., T. G. Northcote, G. F. Hartman, and C. C. Lindsey. 1966. Life histories of two species of catostomid fishes in Sixteenmile Lake, British Columbia, with particular reference to inlet stream spawning. J. Fish. Res. Bd. Canada 23: 1761–1788.

Harris, R. H. D. 1962. Growth and reproduction of the longnose sucker, *Catostomus catostomus* (Forster) in Great Slave Lake. J. Fish. Res. Bd. Canada 19: 113–126.

Hubbs, C. L., and K. F. Lagler. 1958. Fishes of the Great Lakes region. Cranbrook Inst. Sci. Bloomfield Hills, Mich., Bull. 26. 186 p.

Kathrein, J. W. 1951. Growth rate of four species of fish in a section of the Missouri River between Holter Dam and Cascade, Montana. Trans. Am. Fish. Soc. 80: 93–98.

McLain, A. L., B. R. Smith, and H. H. Moore. 1965. Experimental control of sea lampreys with electricity on the south shore of Lake Superior 1953–60. Great Lakes Fish. Comm. Tech. Rept. 10. 48 p.

Olson, D. E., and W. J. Scidmore. 1963. Homing tendency of spawning white suckers in Many Point Lake, Minnesota. Trans. Am. Fish. Soc. 92: 13–16.

Rawson, D. S., and C. A. Elsey. 1950. Reduction in the longnose sucker population of Pyramid Lake, Alberta, in an attempt to improve angling. Trans. Am. Fish. Soc. 78: 13–31.

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Migrations of American Plaice on the Grand Bank and in St. Mary's Bay, 1954, 1959, and 1961

T. K. Pitt

Fisheries Research Board of Canada
Biological Station, St. John's, Nfld.

Pitt, T. K. 1969. Migrations of American plaice on the Grand Bank and in St. Mary's Bay, 1954, 1959, and 1961. J. Fish. Res. Bd. Canada 26: 1301–1319.

Tag returns suggest that the Grand Bank supports a number of relatively sedentary populations of plaice. Apparently no significant migrations between coastal and offshore areas occur.

A total of 4653 American plaice were marked using Petersen disc tags at six localities on the Grand Bank and one in St. Mary's Bay. Total returns ranged from 1.4% for St. Mary's Bay to 41.6% for the east slope of the Grand Bank. Most were recovered less than 30 miles from the tagging site even up to 7 years after tagging.

There was some indication that plaice change their depth location seasonally.

Received November 4, 1968

INTRODUCTION

The only published data on the migrations of American plaice in the northwest Atlantic was by Powles (1965) who gave the results of tagging on the Magdalen Shallows of the western Gulf of St. Lawrence. The largest commercial fishery for American plaice (*Hippoglossoides platessoides* Fabricius) in the northwest Atlantic occurs along the northern and eastern slopes of the Grand Bank in statistical areas 1 to 5 (Fig. 1). In 1967, 180 million lb (82,000 metric tons) were landed mostly by Canadian otter trawlers.

The primary purpose of the tagging programme on which this report is based was to add to the knowledge of the plaice in the Newfoundland area of the northwest Atlantic by estimating the extent of the intermingling between localities and to determine if the area supported a number of discrete populations.

MATERIAL AND METHODS

There were 5 main tagging experiments: St. Mary's Bay, 1954; northern Grand Bank, 1954; south and southeast Grand Bank, 1959; northeast Grand Bank, 1961; and east Grand Bank, 1961 (Table 1 and Fig. 1). In addition, 83 plaice were tagged in September 1960 on the eastern Grand Bank. The latter tagging was terminated when it was thought that the surface temperature (15.5 C) was too high in comparison with the bottom temperature (0.2 C) for good survival.

Petersen disc tags were used for all the experiments. Tags were about 0.5 inches (13 mm) in diameter, 0.036 inches (0.91 mm) thick. One of the discs had the number, along with information for mailing and reward offered, on one side and the address of the Fisheries Research Board Station, St. John's on the other. The other disc was a blank. A variety of colours of tags and blanks was used (Table 1).

Printed in Canada

TABLE 1. Information on tagging localities, and the numbers tagged with the various combinations of upper and lower discs of the Petersen tags. (The letters in the Locality column refer to Fig. 1.)

Locality	Date	Tagging depth		Orange	Yellow	Yellow	White	White	Total tagged
		fath	*m*	Orange	Yellow	White	Yellow	White	
(A) St. Mary's Bay	June 1954	70–86	128–157	449	–	–	–	–	499
(B) N Grand Bank	Oct. 1954	58–64	106–117	1000	–	–	–	–	1000
(C) NE Grand Bank	June 1961	40	73	–	35	40	34	36	145
" " "	"	52	95	–	27	25	29	24	105
" " "	"	64	117	–	248	232	211	201	892
(D) East Grand Bank	Sept. 1960	78–80	143–146	–	83	–	–	–	83
(E) " " "	June 1961	39	71	–	160	156	160	162	638
" " "	June 1961	60	110	–	90	94	70	70	324
(F) SE Grand Bank	July 1959	50	91	778	–	–	–	–	778
(G) S Grand Bank	July 1959	39	71	197	–	–	–	–	197

The tags were attached by .032-inch (0.8-mm) nickel wire. A hypodermic needle was passed through the body of the fish just below the dorsal fin, in the position indicated in Fig. 2. The end of the wire was inserted in the needle and both were withdrawn through the hole simultaneously.

For the 1954 tagging in St. Mary's Bay (A) and on the northeast slope of the Grand Bank (B), and the 1959 tagging on the south (G) and southeast (F) slopes of the bank, only an orange/orange arrangement was used. For the 1960 tagging (83 fish) a yellow/yellow arrangement was used.

In comparing the returns for various colours of tags and blanks for the 1961 tagging, chi-square tests were used employing 2 × 2 contingency tables with the hypothesis that returns are independent of the colour of the tag. Yates' adjustment for continuity was applied. The returns from the two tagging localities were dealt with separately.

None of the combinations in 1961 were significantly different ($P < 0.5$). The percentage returns in the two tagging areas were:

Colour				
Tag	Blank	Avg size (*cm*)	No. tagged	% returned
		Northeast Grand Bank (*C*)		
Yellow	Yellow	46.7	310	40.3
Yellow	White	47.5	297	34.0
White	Yellow	47.2	274	35.0
White	White	46.8	261	34.1
		Eastern Grand Bank (*E*)		
Yellow	Yellow	50.3	250	36.8
Yellow	White	50.2	250	41.6
White	Yellow	49.0	230	41.3
White	White	51.1	232	34.9

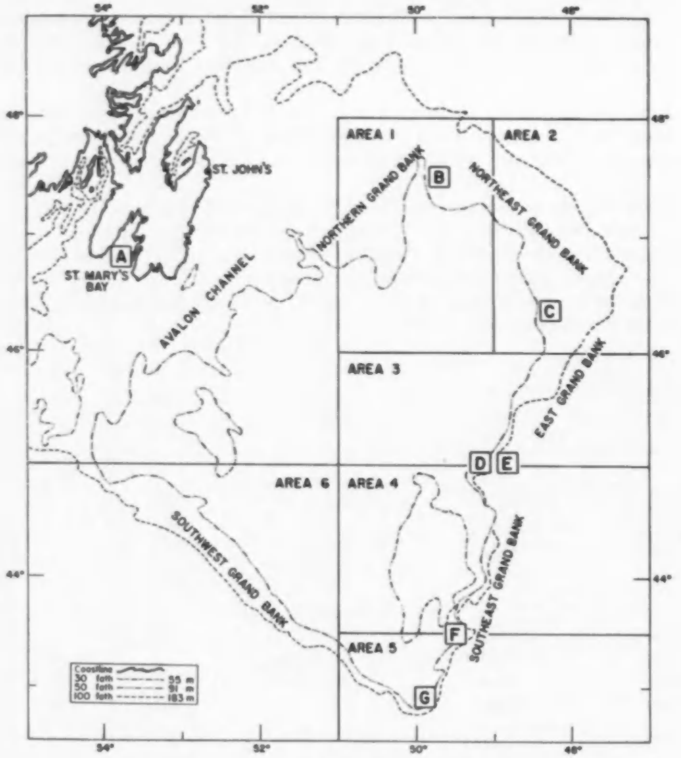
The white/white combination (white tag and white blank) gave the lowest average percentage return for both areas combined. The fish tagged with various tag and blank combinations were of comparable sizes for both areas.

The fish for tagging were caught with an otter trawl using a lined codend with tows of 10–15 min duration. The 1954 tagging was carried out by the 59-ft *Marinus* and the 78-ft *Investigator II* without the use of a tank to hold the fish before tagging. However, for the remainder of the tagging operations, which were carried out on the 168-ft *A. T. Cameron*, the fish were held temporarily in a canvas or a plastic tank prior to tagging.

The smallest fish tagged were from St. Mary's Bay. Although these were above the minimum size limit (20 cm) for the commercial landings (Fig. 3 and 4), the general trend for the industry has been to discard most of the plaice below 30 cm. For the other localities the sizes of the plaice tagged were well within the length range of commercial landings.

ASSESSMENT OF TAG RETURNS

Nearly all of the tags recovered were found in otter trawler catches (150–500 gross tonnage) either on board ship or in the plant cutting room. Rapid handling of the fish after they came from the net probably resulted in some tags being unobserved before the fish were iced below deck. Some of the returns were from deckhands who had little knowledge of the vessel's locality at the time of tag recovery. Locations of tags from the cutting room were plotted only when it was definitely established where the unloading ship had fished and if the catch came from a single locality.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ST. JOHN'S
ST. MARY'S BAY
AVALON CHANNEL
NORTHERN GRAND BANK
NORTHEAST GRAND BANK
EAST GRAND BANK
SOUTHEAST GRAND BANK
SOUTHWEST GRAND BANK
A
B
C
D
E
F
G
Coastline
30 fath 55 m
50 fath 91 m
100 fath 183 m

FIG. 1. Tagging localities, places, and areas named in the text.

The plaice fishery has been primarily Canadian, but trawlers from other countries fish certain localities on the seaward slope of the Grand Bank, especially for cod, and most plaice are discarded. It is likely that the foreign salt-cod fishery captured many tagged plaice which were subsequently discarded. It is also probable that many other tagged plaice were recaptured, but not returned. For example, in 1965–66 the USSR reported 10 million (4400 tons) and 22 million lb (10,000 tons) respectively of "unspecified flounder" (ICNAF, 1965–66). The cost and inconvenience of mailing tags to Canada from Europe also probably resulted in a number of unreported recoveries.

In plotting the locations of tag recoveries (Fig. 6–8) only the returns with reliable information on the fishing position were used. Sometimes it was necessary to accept an approximate position, but this was considered adequate in showing general migratory trends.

Log books placed on board the local trawling fleet gave information on at least 90% of the effort and landings and it was from these data that the relationship between catch and tag returns (Fig. 5) was drawn.

The areas shown in Fig. 1 are arbitrary statistical areas used in connection with the collection of statistics from the commercial fleet by the St. John's Biological Station and these statistical areas are referred to throughout the text. Information on the fishing depth was not always available especially since trawlers frequently towed over a range of depths. This necessitated the

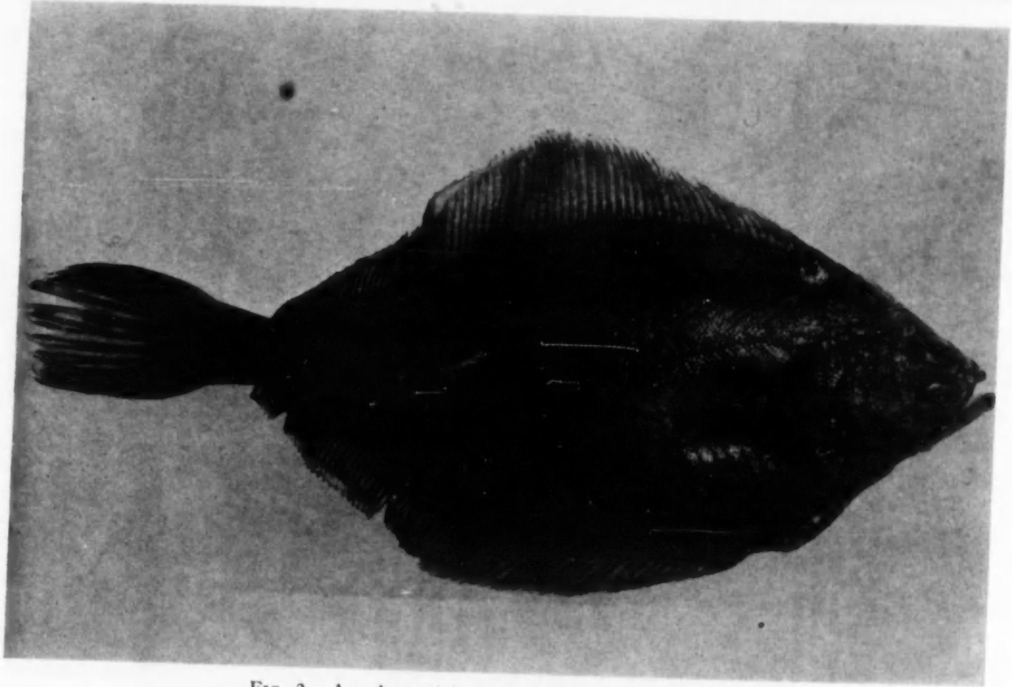

FIG. 2. American plaice with Petersen tag attached.

Pitt — J. Fish. Res. Bd. Canada

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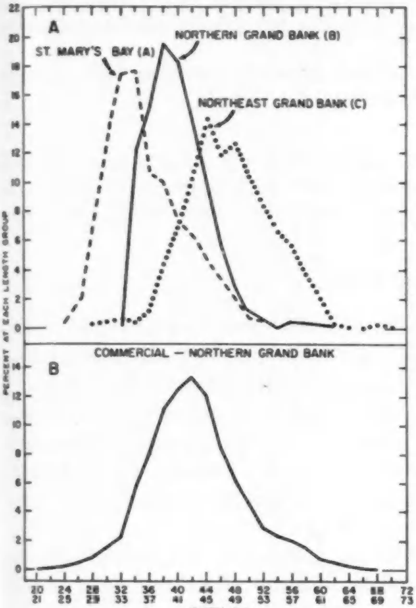
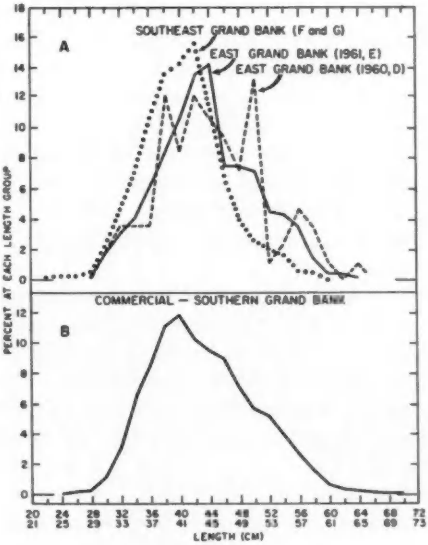


FIG. 3. (A) Length distributions of American plaice tagged at St. Mary's Bay and the northern half of the Grand Bank. (B) Length frequencies of plaice landed in Newfoundland ports from the commercial fishery on the northern half of the Grand Bank (1957–64).

FIG. 4. (A) Length distributions of plaice tagged on the southern half of the Grand Bank. (B) Plaice length frequencies from the commercial fishery in the same area (1957–64).



use of 25-fath (46-m) ranges in showing the depth of recapture (Fig. 10–11). These ranges correspond to those used by the St. John's Biological Station in tabulating catch and effort data from commercial landings.

The only tagging experiments that produced enough returns indicating depth of capture data were the Northeast (C), East (E), and Southeast (F) (Fig. 1).

Distance Travelled

To determine the approximate distances travelled (Fig. 9) a series of concentric circles were drawn of approximately 30, 60, 90, etc. nautical mile radii from the tagging localities and the tags with reliable recovery data counted for each zone. The returns for the year of tagging were not used.

TAG RECOVERY

Relationship between Tag Recovery and the Commercial Catch

More than 70% of the returned tags were from Newfoundland-based trawlers with practically all of the remainder from Nova Scotia (Table 2). This ratio is compatible with the ratio of landings by these two provinces from ICNAF Divisions 3L (northern half of the Grand Bank) and 3N (southern half) for 1959–65 when Newfoundland-based trawlers landed 70.5 and 82.6% of the reported catches of plaice in 3L and 3N respectively and Nova Scotia took 24.6 from 3L and 15% from 3N (ICNAF, 1959–66).

The recovery of tagged fish is obviously related to the fishing intensity. As previously stated, the Newfoundland landings of plaice represent 70–80% of the total landings of that species from the Grand Bank (excluding "unspecified flounder" by the USSR). In assessing the success of the major tagging programmes, the relationship between the landings of Newfoundland trawlers from a number of statistical areas and the per cent of the total tagged that were returned during 3 years after release, is presented in Fig. 5.

In plotting this relationship the exact position of recovery was not always available, so the assumption was made that the trend shown in Table 3 held also for the tags with insufficient recovery data. Thus it was assumed that: (*a*) a majority of the fish tagged at B (Fig. 1) (northeast slope, Grant Bank) were recaptured in statistical areas 1 and 2; (*b*) those tagged in 1961 at C were recaptured in areas 2 and 3; (*c*) those tagged at D and E were recovered in areas 3 and 4; and (*d*) the fish from F and G were recovered in areas 4 and 5.

The relationship between the number of tags recovered in the 3 years after tagging and the total landings for the 3 years was especially good for the two northern tagging sites (Fig. 5B). The recoveries for the 1959 tagging on the south and southeast slopes (F and G) were relatively high in proportion to the landings from this area (5A). Only 83 fish were tagged in 1960 (D) and these were probably too few to give reliable percentages.

Returns for the Various Tagging Experiments

(*A*) *St. Mary's Bay* (*Fig. 6A*) — The returns from this tagging were small with only 7 (1.4%) tags returned from 499 released (Table 4). One tag

Table 2. Percentages of recovered tags that were taken by trawlers of different countries for the major Grand Bank tagging programmes. (Letters refer to areas in Fig. 1.)

Tagging date and locality			Canada		France: St. Pierre	Spain	Other European	Total returned
			Nfld.	N. S.				
(B)	Oct. 1954	N Grand Bank	63.2	36.8	–	–	–	58
(C)	June 1961	NE Grand Bank	75.1	19.9	2.0	1.2	1.5	411
(E)	June 1961	E Grand Bank	73.8	25.3	0.6	–	0.3	372
(F & G)	July 1959	SE & S Grand Bank	78.4	19.3	1.8	–	0.5	219

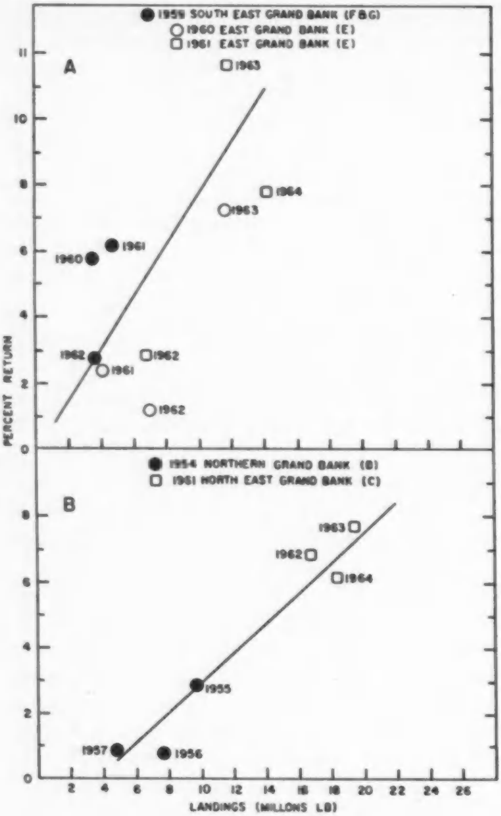


FIG. 5. Relationship between tag recoveries and the commercial landings for 3 years after tagging for (A) the southern and (B) the northern parts of the Grand Bank.

was recovered on the northeast slope of the bank but the remainder were taken from localities 60 miles (nautical) or less from the tagging site.

(*B*) *Northern Grand Bank 1954* (*Fig. 6A*) — Fifty-eight tags were returned from 1000 tagged (Table 4), with about a half of these being recovered the year after tagging. Most of the returns with reliable information came from the same statistical area in which the tagged fish were released (Table 3). Most were recovered within a 30-mile radius of the release site (Fig. 9, tagging area B).

(*C*) *Northeast Grand Bank* (*Fig. 7*) — For the three tagging depths (Table 4) 32.7% of the tags were returned. The recaptures from the 40-fath (73-m) tagging were 23.6% but for the other two depths the recapture rate was approximately 37%. A greater percentage of plaice tagged at 64 fath (117 m) were recovered within a 30-mile radius than for those at 40 and 50 fath (73 and 95 m) (Fig. 9, tagging area C). One fish released at 40 fath (73 m)

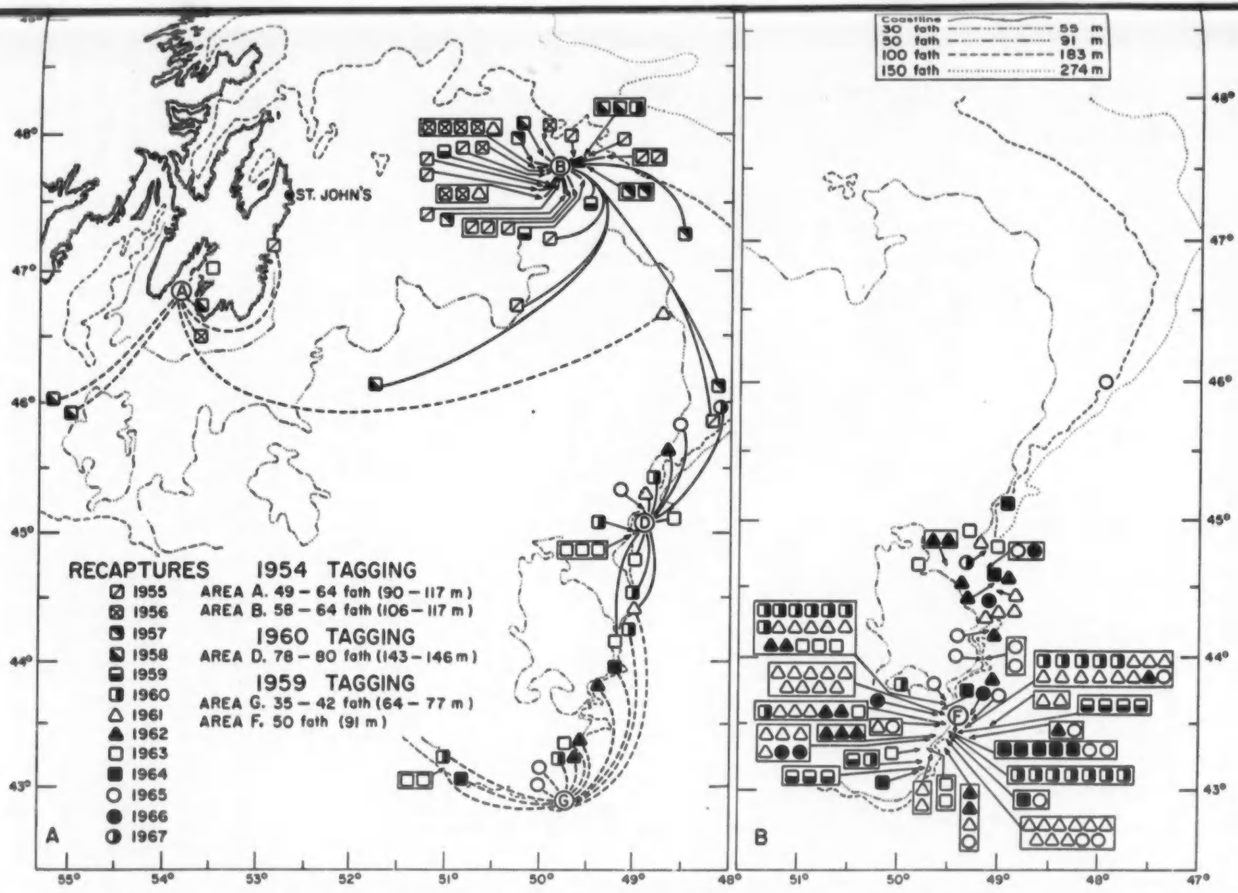
TABLE 3. Percentages of recovered tags that were returned from the various Grand Bank statistical areas described in the text. (The letters refer to the tagging locations in Fig. 1.)

Tagging area	Recovery areas							Total recovered
	1	2	3	4	5	6	Unknown	
B(1)[a]	53.4	3.0	3.0	–	–	–	29.5	58
C(2)	7.4	38.8	12.7	1.5	–	–	39.6	411
E(4)	0.6	2.8	14.2	44.6	0.3	–	37.5	372
F & G(5)	–	–	0.9	29.0	40.0	0.5	28.1	219

[a] Statistical area in parentheses.

TABLE 4. Percentages of tags recovered in various years for the various tagging experiments. (The letters in the Locality column refer to the tagging areas in Fig. 1.)

Tagging information					Years after tagging										Total
		Depth													
Locality	Date	*fath*	*m*	No.	0+	1	2	3	4	5	6	7	8	9	%
(A) St. Mary's Bay	June 1954	49–64	90–117	499	–	0.2	0.2	–	0.6	–	–	0.2	–	0.2	1.4
(B) N Grand Bank	Oct. 1954	58–64	106–117	1000	–	2.6	0.7	0.9	0.5	0.4	0.3	0.3	–	0.1	5.8
(C) NE Grand Bank	June 1961	40	73	145	1.4	2.8	4.8	2.8	6.9	1.4	3.5	–	–	–	23.6
" " "	" "	52	95	105	5.7	4.8	11.4	4.8	5.7	3.8	1.0	–	–	–	37.2
" " "	" "	64	117	892	7.5	7.7	8.1	6.8	4.6	1.9	0.6	–	–	–	37.3
(D) E Grand Bank	Sept. 1960	78–80	143–146	83	4.8	2.4	1.2	7.2	3.6	4.8	3.6	1.2	–	–	28.8
(E) " " "	June 1961	39	71	638	3.5	3.1	12.9	8.9	8.0	3.6	1.6	–	–	–	41.6
" " "	June 1961	60	110	324	3.4	2.5	10.2	5.9	4.6	4.6	1.2	–	–	–	32.4
(F) SE Grand Bank	July 1959	50	91	778	1.3	4.2	7.5	3.1	2.0	2.7	3.1	0.9	0.5	–	25.3
(G) S Grand Bank	July 1959	39	71	197	–	2.6	1.0	1.5	2.0	2.0	1.0	–	0.5	–	10.6


RECAPTURES
▨ 1955
⊠ 1956
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⊟ 1959
◧ 1960
△ 1961
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□ 1963
■ 1964
○ 1965
● 1966
◐ 1967
1954 TAGGING
AREA A. 49 – 64 fath (90 – 117 m)
AREA B. 58 – 64 fath (106 – 117 m)
1960 TAGGING
AREA D. 78 – 80 fath (143 – 146 m)
1959 TAGGING
AREA G. 35 – 42 fath (64 – 77 m)
AREA F. 50 fath (91 m)
ST. JOHN'S
A
B
D
G
F
Coastline
30 fath 55 m
50 fath 91 m
100 fath 183 m
150 fath 274 m
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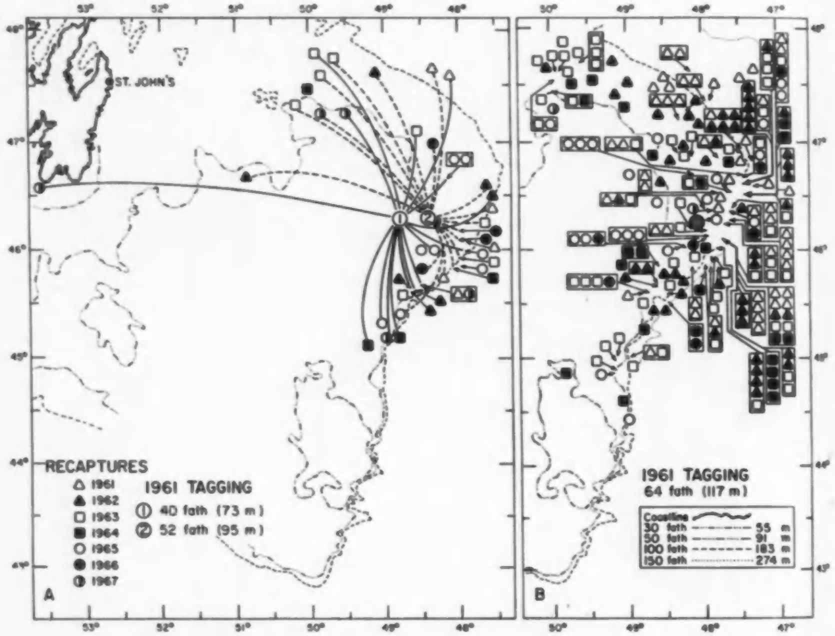
FIG. 6. (A) Recoveries from plaice tagged at areas A, B, D, and G in 1954, 1959, and 1960. (B) Recoveries from plaice tagged at 50 fath (91 m) on the southeast slope of the Grand Bank (tagging area F) in 1959. (Each symbol represents a tag recovery.)

was caught in 17 fath (31 m) near St. Mary's Bay (Fig. 7A). Most of the returns with reliable information were from the same statistical area in which they were released (Table 3).

(*D*) *East Grand Bank, 1960* (*Fig. 6A*) — Of the 83 tags released 29% were returned (Table 4). This apparently was a successful tagging in spite of a large difference between surface and bottom temperatures at the time of tagging, as previously mentioned.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ST. JOHN'S
RECAPTURES
△ 1961
▲ 1962
□ 1963
■ 1964
○ 1965
● 1966
◑ 1967
1961 TAGGING
① 40 fath (73 m)
② 52 fath (95 m)
A
B
1961 TAGGING
64 fath (117 m)
Coastline
30 fath 55 m
50 fath 91 m
100 fath 183 m
150 fath 274 m

FIG. 7. Recoveries from plaice tagged in 1961 on the northeast slope (tagging area C) of the Grand Bank, (A) at 40–52 fath (73–95 m) and (B) at 64 fath (117 m). (Each symbol represents a tag recovery.)

(*E*) *East Grand Bank, 1961* (*Fig. 8*) — Approximately 42% of the tags were returned from 638 plaice tagged at 39 fath (71 m) and 32% from 324 tagged at 60 fath (110 m) (Table 4). A majority of the returns were from localities less than 30 miles from the tagging locality (Fig. 9, tagging area E). The highest percentage of the tags (Table 3) came from the slope of the bank south of the tagging locality (Fig. 8). Three tags, however, were recovered from the northern slope of the bank at about 47°30′N and 50°00′W and two along the northwest slope (Fig. 8A). As with the previous two experiments most tags were taken in the same statistical area in which they were tagged (Table 3). The tagging here occurred on the boundary between areas 3 and 4 (Fig. 2).

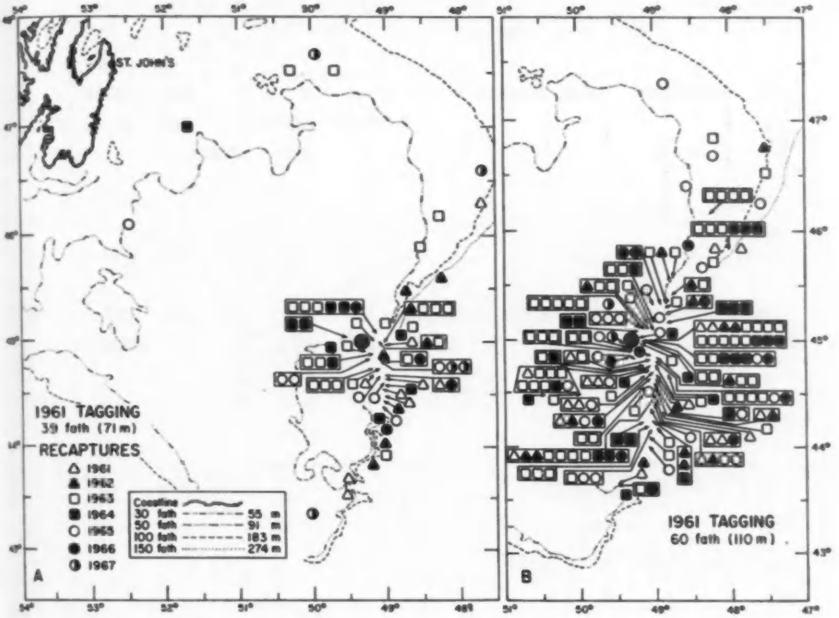
ST. JOHN'S
1961 TAGGING
39 fath (71 m)
RECAPTURES
△ 1961
▲ 1962
□ 1963
■ 1964
○ 1965
● 1966
◑ 1967
Coastline
30 fath 55 m
50 fath 91 m
100 fath 183 m
150 fath 274 m
A
1961 TAGGING
60 fath (110 m)
B
54° 53° 52° 51° 50° 49° 48° 47°
47° 46° 45° 44° 43°

FIG. 8. Recoveries from plaice tagged in 1961 on the eastern slope (tagging area E) of the Grand Bank, (A) at 60 fath (110 m) and (B) at 39 fath (71 m). (Each symbol represents a tag recovery.)

(*F*) *Southeast Grand Bank, 1959* (*Fig. 6B*) — Approximately 25% of the tags were returned from the 778 fish tagged (Table 4). Most of the recaptures were made less than 30 miles from the tagging area (Fig. 9, tagging area F). One tag was recovered from the southwest slope of the bank and 40% of the recoveries were in the same statistical area (5) as the tagging site; however, 29% of the recovered tags came from the adjacent area to the north (area 4) (Table 3).

(*G*) *South Grand Bank, 1959* (*Fig. 6A*) — Of the 197 tags 10.6% were returned (Table 4). Most were reported from localities less than 60 miles from the tagging site. Four tags were recovered on the southwest slope of the Grand Bank.

DEPTH OF RECAPTURE OF TAGGED PLAICE

Northeast Grand Bank (*tagging area C*) — The seasonal depth distribution of tag returns corresponded roughly with that of the commercial fishing effort in the period (Fig. 10); 38.8% of the recovered tags were returned from statistical area 2 and 12.7% from area 3 and the remainder from areas 1 and 4 (Table 3). There was very little fishing for plaice during the first quarter and a correspondingly low percentage of tag returns. In the second quarter most

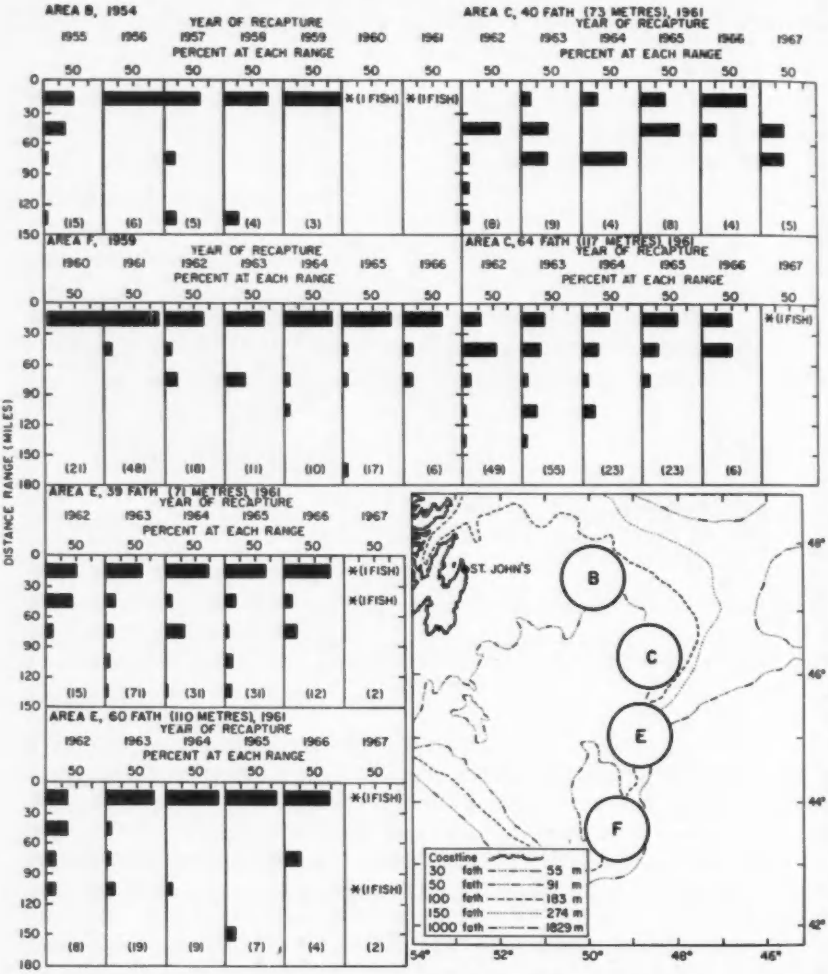

FIG. 9. Percentages of recovered tags that were taken in various years within various distances for a number of tagging experiments. Each circle on the map indicates a radius of approximately 30 nautical miles from the tagging position.

of the tag recoveries and the effort were at 51–75 fath (93–137 m). However, during the last half of the year most of the recoveries came from 76–100 fath (138–183 m) and the commercial effort during the latter period was generally concentrated at greater depths than during the first half of the year.

East Grand Bank (*tagging area E*) — The seasonal patterns of recovery from the two depths appeared to be similar (Fig. 11). As with the northeast Grand Bank very little fishing took place during January–March and for

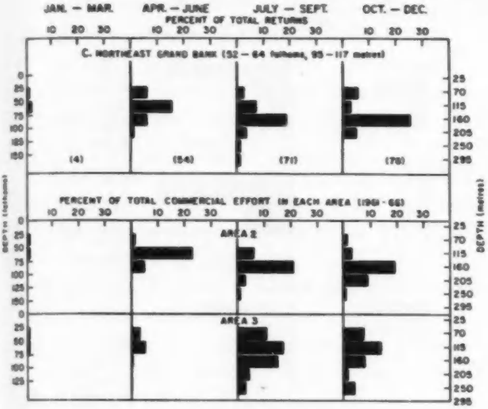

FIG. 10. Percentages of recovered tags that were taken quarterly at various depths for tagging carried out in 1961 (tagging area C) and the depth distributions of the effort (in hours) of the Newfoundland based commercial fleet for 1961–66. (Areas refer to Fig. 2.)

areas 3 and 4 the second quarter effort was also low. During July–September the greatest percentage of returns was from 51–75 fath (93–137 m) although a large proportion of the effort in area 4 (44.6% return) was at 26–50 fath (48–91 m). In area 3 (14.2% return), however, there was considerable effort below 50 fath (91 m). In the fourth quarter the largest percentage of the tags were recovered from 26–50 fath (48–91 m) corresponding to the effort pattern.

Southeast and south Grand Bank (*tagging area F*) — Of the total tag returns, 29% were from area 4 and 40.0% from area 5 (Table 3). Except for the third quarter, most of the tags were taken from shallow water with the pattern of tag recovery corresponding roughly to the commercial fishing effort (Fig. 11). As with the previous area, during July–September, a slightly greater percentage of tags were recovered from 51–75 fath (93–137 m) than at the other depths although a large proportion of the effort was at 26–50 fath (48–91 m).

DISCUSSION

TAG RETURNS

Except for the fact that the white/white arrangement on the average gave a lower percentage return than other combinations, there appeared to be no real difference in returns between colours of tag used. A white disc on the pale lower side of a flatfish would obviously not show up as well as a contrasting colour. Unfortunately orange and red tags were not involved in the colour comparison.

The relationship of tag returns to landings for the southern half of the Grand Bank (Fig. 5) was much more irregular than for the northern. The 1960 and 1961 returns from the 1959 tagging (tagging areas F and G, Fig. 2) were high in proportion to landings. Plaice populations in this area are confined to a relatively small locality. The slope of the bank beyond the 40-fath (73-m) contour is very steep so that most of the plaice are probably concentrated at

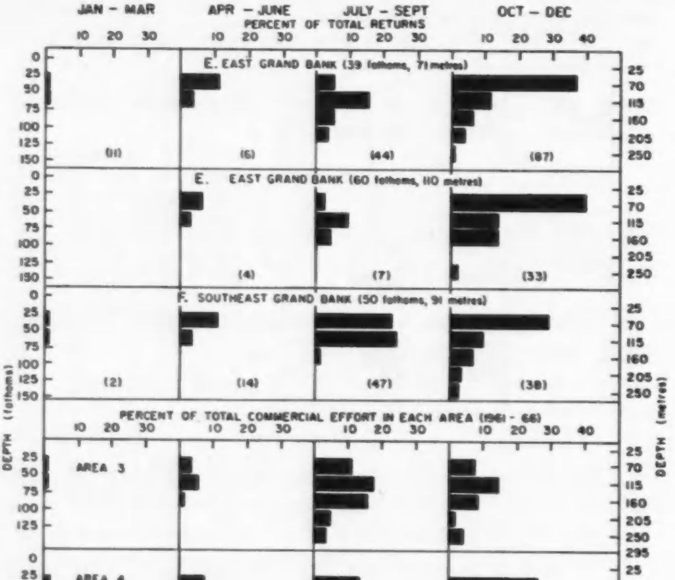

FIG. 11. Percentages of recovered tags that were taken quarterly at various depths for tagging carried out in 1961 (tagging area E) and in 1959 (tagging area F) and the depth distributions of the fishing effort (in hours) by the Newfoundland based commercial fleet for 1961–66. (Areas refer to Fig. 2.)

35–60 fath (64–110 m) and the Southeast Shoal to the north of this area presents a barrier to any movement northward from this locality across the bank. A relatively high fishing mortality with a correspondingly high tag return in the years immediately after tagging is borne out by age-frequency data (Pitt, 1967a and unpublished data).

In St. Mary's Bay and adjacent areas there was very little fishing for plaice until the mid-1960's. This probably accounts for the relatively small tag returns here.

MIGRATIONS

Generally speaking, American plaice tagged on the Grand Bank moved very little. Most of the recoveries, even up to 7 years after release, were made less than 30 miles (48 km) from the release locality.

There was no evidence of substantial migrations between bank areas and Newfoundland inshore localities. Up to 1964, however, inshore boats did not land plaice, so it was probably too late to recover any of the 1954 tags from the northern part of the Grand Bank if indeed any tagged plaice did move inshore from the Grand Bank. Only one tag from the 1961 tagging was recovered inshore.

Most fish migrations are associated with spawning or feeding patterns, with changes in the environmental conditions or probably a combination of all three. A variety of temperature and feeding conditions necessitating very little movement is available to the fish on the slopes of the Grand Bank. Although plaice make limited diurnal movements (Pitt, 1967b) it is doubtful if they pursue pelagic food species for great distances. Therefore, large mass migrations of this species apparently do not take place.

Plaice appear to spawn on most of the Grand Bank and in the bays and coastal areas (Pitt, 1965) so that there are no large spawning migrations. The shallow areas of the bank probably serve as a nursery ground for the Grand Bank population with the young fish moving deeper as they grow larger in size. The locations of inshore nursery grounds are not known.

The fact that the commercial fleet vary their effort seasonally with respect to depth suggests that plaice do change their depth locality throughout the year. Tagging from different depths did not indicate a different recovery pattern. Note that all depth data were grouped in 25-fath (46-m) categories so that slight movements did not show up in the tabulations. Little fishing effort occurred from December to early April so that we have little knowledge of the fish's location during this period. Actually, seasonal change in depth distribution of up to 75 fath (137 m) on the slope of the bank would not, as a rule, entail great horizontal movement.

Powles (1965) found that Magdalen Shallows American plaice migrated in late fall from shallow inshore feeding grounds to deeper offshore areas; however, this did not entail a very great movement. Milinsky (1944) indicated that the Barents Sea variety of ***Hippoglossoides platessoides*** makes fairly extensive spawning migrations. Royce et al. (1959) showed that yellowtail off the New England Coast migrate up to 200 miles annually and there are many records of extensive migration by other species of flatfish.

Figure 12, a summarization of the results of the various tagging programmes, shows that for each area there was a relatively slight dispersal of plaice from the tagging area with no great intermingling from widely separated areas of the bank. Therefore, the division of the Grand Bank into the arbitrary sampling areas (Fig. 1 and 12) for various problems in the population dynamics of this species is corroborated by tagging.

Area 6 (southwest Grand Bank) apparently receives very few recruits from other areas, but here could be migration from the area. St. Mary's Bay probably supports a resident population.

The Grand Bank apparently has a large number of relatively sedentary stocks of this species so that if seasonal schooling occurs, there are numbers

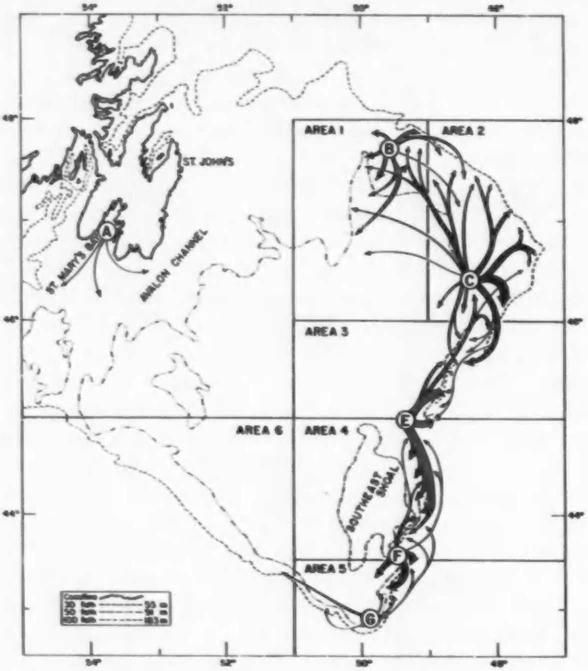

FIG. 12. Summary of the general migratory patterns.

of spawning or feeding schools rather than a few large schools that would be vulnerable to an intensive fishing effort. This probably prevents drastic reduction in the population by an intensive fishing effort. The result, however, is possibly a gradual reduction in the catch per unit effort over the whole bank with recruitment dependent on the growth of the younger fish which are moving from the shallow areas.

ACKNOWLEDGMENTS

The assistance of Mr C. A. Rose in the tagging operations and in preparation of the data for publication is gratefully acknowledged. Suggestions for improvement were provided by several members of the scientific staff of the St. John's Station.

REFERENCES

INTERNATIONAL COMMISSION FOR THE NORTHWEST ATLANTIC FISHERIES. 1959–66. Statist. Bull. 9–15.

MILINSKY, G. I. 1944. [On the biology and fisheries of the long rough dab of the Barents Sea.] Tr. Poliarnogo N.-I. Inst. Morsk. Rybn. Khoz. Okeanog. Im. N.M. Knipovicha (PINRO) 8: 388–415. (In Russian with English summary.)

PITT, T. K. 1965. Sexual maturity and spawning of the American plaice, *Hippoglossoides platessoides* (Fabricius) from Newfoundland and Grand Bank areas. J. Fish. Res. Bd. Canada 23: 651–671.

1967a. Age and growth of American plaice (*Hippoglossoides platessoides*) in the Newfoundland area of the Northwest Atlantic. J. Fish. Res. Bd. Canada 24: 1077–1099.

1967b. Diurnal variations in the catches of American plaice (*Hippoglossoides platessoides* Fab.) from the Grand Bank. Intern. Comm. Northwest Atlantic Fish. Res. Bull. 4: 53–58.

POWLES, P. M. 1965. Life history and ecology of American plaice (*Hippoglossoides platessoides* F.) in the Magdalen Shallows. J. Fish. Res. Bd. Canada 22: 565–598.

ROYCE, W. F., R. J. BUTLER, AND E. D. PREMETZ. 1959. Decline of the yellowtail flounder (*Limanda ferruginea*) off New England. U.S. Fish Wildlife Serv., Fish. Bull. 146: 169–267.

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Some Aspects of the Ecology and Life Cycle of Three Species of Subtidal Sand-Burrowing Amphipods (Crustacea: Haustoriidae)[1]

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SAMEOTO, D. D. 1969. Some aspects of the ecology and life cycle of three species of subtidal sand-burrowing amphipods (Crustacea: Haustoriidae). J. Fish. Res. Bd. Canada 26: 1321–1345.

The life cycles and some aspects of the ecology of the subtidal haustoriid amphipods *Acanthohaustorius millsi* Bousfield, *Parahaustorius longimerus* Bousfield, and *Protohaustorius deichmannae* Bousfield, found on Cape Cod, Massachusetts, are described. All species are annual breeders, the first two species having one brood of eggs and the last species more than one brood. The first ovigerous females appear when the water temperature reaches 5.5–10 C. All species migrate into the sands just below the low tide zone in May and remain there until late August when they start to migrate into deeper waters. The growth of populations of the three species found at Nobska Beach and Barnstable Harbor are discussed.

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INTRODUCTION

THE HAUSTORIIDAE is a family of amphipods that has been recently enlarged by the addition of a number of new species from the east coast of North America (Bousfield, 1962, 1965). These amphipods are sand-burrowing animals that feed by filtering fine food particles from the interstitial water of sands. A complete description of their morphology, phylogeny, evolution, and zoogeography was given by Bousfield (1965), but little was known of their life cycles and ecology. This study was therefore undertaken to provide this information.

The species studied were found on Cape Cod, Massachusetts. They were: (1) *Protohaustorius deichmannae* Bousfield, a species found subtidally in shallow waters, with a range from central Maine to Georgia. It is phylogenetically the most primitive of the species studied. (2) *Parahaustorius longimerus* Bousfield, a species found subtidally from northern Florida to Cape Cod. This species is common from surf-exposed and semi-protected sandy coasts to the offshore banks. (3) *Acanthohaustorius millsi* Bousfield,

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mainly a subtidal species that has been found to a depth of 27 fath. It is the most common species of Haustoriidae found on Cape Cod.

Two areas on Cape Cod were selected for study (Fig. 1). They were Nobska Beach in the town at Woods Hole on the southern tip of the Cape and Barnstable Harbor on Cape Cod Bay.

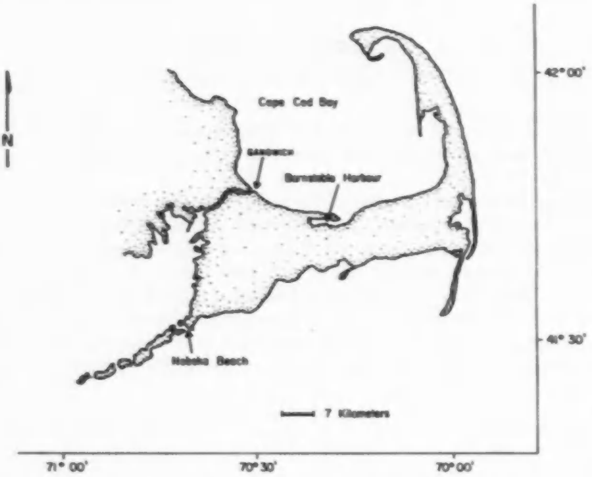

FIG. 1. Cape Cod, Massachusetts showing the locations of the study areas.

METHODS

All methods used were the same as those described in the Black Beach – Sippewissett study (Sameoto, 1969) with the exception of the mesh size used for screening the sand. A 0.5-mm mesh was used for the Nobska Beach sand whereas a 1.0-mm or 0.5-mm mesh was used for the Barnstable Harbor samples depending on the coarseness of the sand. Samples were taken every five paces along two transects at Nobska Beach starting at the low tide line, and random locations on a sand bar at Barnstable Harbor.

All dry weight versus body length curves were determined by the least squares method of finding a polynomial regression.

COLLECTION AREAS

NOBSKA BEACH

Nobska Beach is approximately one third of a kilometer long. To the southeast it is protected by a rocky point, which makes the waters adjacent to this point considerably calmer than those farther west along the beach. For this reason two sampling transects were established, the first in the sheltered waters to the immediate west of the point and the second in the more exposed middle zone of the beach about 200 m from the first transect.

A zone of poorly sorted coarse sand was always present at the low water line of the beach (Table 1), with a 1-m deep trough seaward of this sand

TABLE 1. Mean sand grain sizes for the 90, 50, and 10 percentiles and degrees of sorting for sands at Nobska Beach and Barnstable Harbor, and mean organic contents of the sands at Nobska Beach.

Sample site	Mean grain size (mm) Percentile 90	50	10	DDϕ[a]	Mean organic content ± SD (%)	No. of samples
		Nobska				
Transect 1						
Low water line	2.020±0.060[b]	0.912±0.044	0.340±0.037	1.2	0.187±0.031	3
Shore side of barrier bar	0.376±0.031	0.279±0.021	0.217±0.011	0.4	0.250±0.028	9
Top of barrier bar	0.360±0.034	0.271±0.020	0.208±0.014	0.4	0.250±0.025	9
Transect 2						
Low water line	1.896±0.350	1.110±0.260	0.350±0.040	1.1	0.195±0.036	4
20 m from low water line	1.110±0.080	0.674±0.060	0.374±0.035	0.7	0.254±0.020	4
		Barnstable Harbor				
				DDϕ	No. of samples	
Coarsest sand	0.960	0.575	0.237	1.0	35	
Finest sand	0.365	0.271	0.187	0.5		
Most common sand	0.482	0.304	0.222	0.3		

[a] See Sameoto (1969).
[b] One standard deviation of the mean.

Beyond the trough there was a barrier bar of fine sand that extended to the end of the first transect, a distance of approximately 40 m. The barrier bar was not built up until the middle of June, and was washed away in November. The mean depth of water at low tide at the end of the first transect was about 1.25 m, which was the maximum depth at which collection was possible without a boat.

During low spring tides the bar was exposed to the air, but was always water-saturated and wave-washed. A zone of black sand was present in the bar 6 cm below the surface in the winter and spring and 2 or 3 cm from the surface in the summer. Brafield (1964) showed the oxygen level in areas of grey sand 4 cm below the surface and having 18.5% of the grains 0.25 mm in diameter or less to be 10.5–12.0% of that in air-saturated water. Since over 20% of the sands of Nobska Beach were less than 0.25 mm in diameter, it is probable that the oxygen levels were near the levels reported by Brafield.

The temperatures of the sand at low tide during the collection periods are given in Fig. 2 and the high tide water temperatures in Fig. 3. The salinity range during the year was 31.2–31.7‰. With the exception of the coarse sand at the low tide line the organic content of the sands (Table 1) did not vary significantly from station to station or with different collection times.

An important factor on the beach was the wave action which prevented a barrier bar from forming at transect two. The sands at this transect were poorly sorted and generally coarser than those of transect one (Table 1).

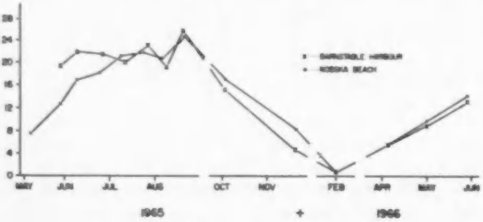

FIG. 2. The sand temperatures at low tide for Nobska Beach and Barnstable Harbor over a 12-month period.

BARNSTABLE HARBOR

A large permanent sand bar in front of the Yacht Club in the town of Barnstable was selected for study for the following reasons: (1) The three species of Haustoriidae studied at Nobska Beach were found also at Barnstable Harbor; therefore it was possible to look for changes in the ecology and life cycles of different populations of these species. (2) The sand bar was exposed to very strong tidal currents, large tidal fluctuations, and to the air for up to 2 hr during low spring tides. The area had a lower yearly water temperature range than Nobska Beach (Fig. 3). This made the region a different physical environment than Nobska Beach. Therefore it was possible to study changes in the population structure of the Haustoriidae group, *P. longimerus*, *A. millsi*, and *P. deichmannae*, that may have resulted from the different environment.

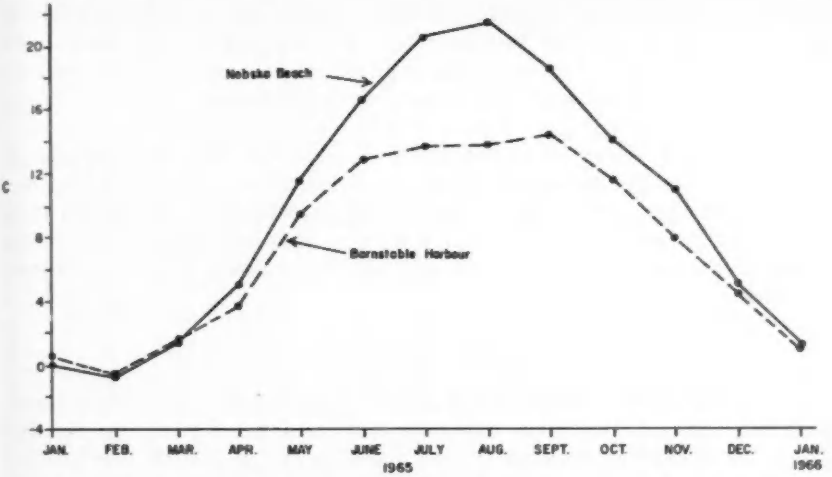

FIG. 3. Monthly mean water temperatures at high tides in the two study areas, January 1965–January 1966. Nobska Beach temperatures taken at Woods Hole oceanographic dock (½ mile from beach). Barnstable Harbor temperatures taken at Sandwich.

The average tidal range in the harbor was 2.88 m; the salinity of the water ranged from 30.6 to 30.9‰ for the entire summer. The speed of the tidal current over the sand bar measured by the method described by Sameoto (1969) was 1 m/sec at 2½ hr before low tide. This current caused extensive sand drifting and erosion, but the sand bar maintained a stable shape and size for the duration of the study. The sand bar was exposed to the air for up to 2 hr during low spring tides and only for ½–1 hr during neap tides. During July and August, the sun would occasionally heat the sands to temperatures of 26 C. The sand temperature at low tide is given in Fig. 2. High sand ridges of the bar were above the water table for a short time during the spring tides, but generally the sands remained saturated at all times during low tide.

The sand bar contained a mosaic of sand grain sizes ranging from a median size of 0.575 to 0.271 mm in diameter (Table 1). The mean per cent weight of the organic content of the sand was 0.300 ± 0.041%, with a range of 0.225–0.368%. This was significantly higher than the organic content of Nobska Beach sand ($t = 4.50$, df $= 46$; $P < .01$). No correlation was found between the grain size and the organic content. To find if the higher organic content of the sand at Barnstable was due to a greater amount of algae in the sand, a measure of the plant pigments at the two locations was made. This was done by removing the plant pigment from several 10-ml samples of wet sand with 20 ml of 85% acetone. The mixture was shaken vigorously for several minutes, then stored in the dark for 24 hr with regular shaking. An absorption spectrum was determined for all wave lengths from 750 to 350 μ. There was no noticeable difference in the amount of absorption between the Nobska Beach and Barnstable Harbor samples. This did not mean that no difference existed at other times of the year. But since there was a significant difference in the total organic content of the two areas at all times of the year, it seems unlikely from the above results that living plants in the sand were responsible for the difference.

Black sand was found approximately 3 cm below the surface of sand at all times. Since the percentage of the sands less than 0.25 mm in diameter was greater than 10% in sands containing Haustoriidae, the animals must have lived in low oxygen conditions similar to those at Nobska Beach in the summer. The regions with no black sand were very coarse (sands of 2.0 mm) and did not contain amphipods.

NOBSKA BEACH SPECIES

Acanthohaustorius millsi, *Protohaustorius deichmannae*, and *Parahaustorius longimerus*, plus two species of *Bathyporeia*, *B. quoddyensis* and an unidentified species, were found in the sands of both transects, but no animals were found in the coarse sand at or above the low water line. The number of animals in the sands increased as the water warmed in the spring, then decreased in the fall with the drop in water temperature (Table 2, Fig. 4). The most probable explanation for the decrease in the fall was a movement into deeper water as the temperature dropped. Large numbers of adults of all three species reappeared in the spring during the breeding season. Since there was no evidence of a fall or winter generation these were likely the same animals that were present in the late summer and fall.

There were significantly higher densities of *A. millsi* ($t = 9.01$, df $= 10$; $P = <.01$) and *P. deichmannae* ($t = 3.62$, df $= 10$; $P = <.01$) in transect 1 than in transect 2. There was no significant difference in the density of *P. longimerus* in the two transects. The density of *Bathyporeia* was greater in transect 2 ($T = 2.86$, df $= 10$; $P = < .05$).

LIFE CYCLE AND FECUNDITY OF *ACANTHOHAUSTORIUS MILLSI*

The length of the life cycle of *A. millsi* was between 12 and 17 months. The last members of the old generation died at least before October (Table 3). In 1965 the ovigerous females appeared in early May when the water temperature was 7.5 C. On May 5, 1966 the water temperature was 9.7 C and 54% of the females were ovigerous, whereas on May 7, 1965, the water temperature was 7.5 C and only 12.7% of the females were ovigerous. No ovigerous females were found in the April samples, when the temperature was 6.5 C. The number of ovigerous females dropped after the middle of July with very few being found in August.

The sex ratio before the first young were released was 1:1, but soon changed in favour of the males as the post-ovigerous females died (Table 3). The difference between the sex ratios for the months of October and November was probably due to the difficulty of sexing the juveniles and did not reflect a

TABLE 2. Mean numbers of various species of amphipods per m² in sands of Nobska Beach on various dates, 1965 and 1966 (N1 = transect 1; N2 = transect 2).

Date	*A. millsi*		*P. longimerus*		*P. deichmannae*		*Bathyporeia* sp.		No. of samples	
	N1	N2	N1	N2	N1	N2	N1	N2	N1	N2
					1965					
May 22	900±180[a]	75±45	225±90	45±30	150±135	0	75	75	6	5
June 7	1440±165	75±15	210±210	15±15	285±390	1	30±45	165±15	7	5
June 21	2475±900	195±15	405±165	120±120	1005±1080	60±45	570±675	1230±105	7	5
July 6	1650±1365	208±60	375±270	360±270	1290±2535	180±45	510±735	1035±1155	8	5
July 20	1800±795	240±60	660±375	555±345	825±1140	75±75	465±375	540±480	6	6
Aug. 4	2280±1275	150±75	525±180	375±300	1890±2115	150±255	375±435	300±300	6	5
Aug. 18	945±285	150±75	630±240		2145±2460		375±945		5	
Oct. 1	375±285		60±15		60±30		60		5	
Nov. 19	855±315		375±15		105±75		900		7	
					1966[b]					
Feb. 2	135		0		0		0		10	
Apr. 4	15		1.5		10		15		10	
May 5	135		60		3		105		10	
May 27	195		90		60		240		10	

[a] One standard deviation of the mean.
[b] Means of 10 samples combined.

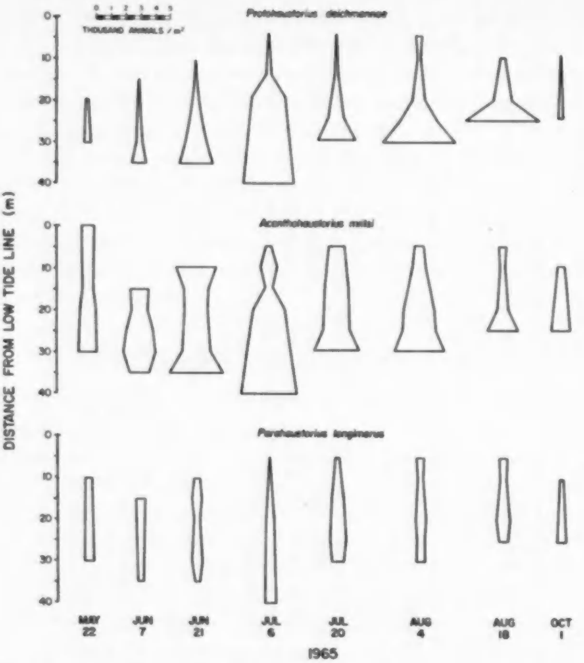

FIG. 4. Densities (per m²) of three species of Haustoriidae according to distance from low tide line along transect 1 at Nobska Beach, May–October 1965.

true fluctuation in the ratios. In February, when the animals had matured and sex determination became easier, the ratio was once more very close to 1:1.

Table 3 suggests that the females produced only one brood and then died. If the number of broods per female were large, the percentage of ovigerous females would have reached a high plateau for a longer period than the incubation time for one brood, rather than a sharp peak and then a steady decline in the percentage of ovigerous females, as was the case. An example of a plateau in the percentage of ovigerous females was seen with *Neohaustorius biarticulatus* (Sameoto, 1969), where the percentage and number of females with eggs remained relatively constant for most of the summer.

A linear relationship was found (Fig. 5) between the body weight of the females and the number of eggs, the largest brood being carried by the largest female. The equation:

$$Y = 3.52 + 1.32\ X$$

means that 1.32 eggs were added to the brood for every 1-mg increase in body

TABLE 3. Percentages of collections of three species of Haustoriidae from Nobska Beach on various dates, 1965 and 1966, that were adults, of the adults that were females, and of the collections that were females in various stages of maturation.

	1965										1966			
	May 7	May 22	June 7	June 21	July 6	July 20	Aug. 4	Aug. 18	Oct. 1	Nov. 19	Feb. 2	Apr. 4	May 5	May 27
						A. millsi								
No. of specimens	101	337	490	625	568	601	377	401	131	210	95	14	88	127
% adults	97.1	99.1	99.2	84.4	57.6	60.3	37.7	27.7	34.4	73.0	100	100	100	100
% adults that were females	56.1	50.0	41.1	34.6	34.6	32.3	28.9	36.0	27.3	57.6	48.4	50.0	32.0	38.6
% ♀$_e$[a]	6.9	31.7	33.1	14.0	12.8	7.1	0.7	0.9	0	0	0	0	17.0	35.4
% ♀$_b$	0	0	1.4	12.6	3.9	3.8	1.5	0.9	0	0	0	0	3.4	0
% ♀	47.5	17.8	6.3	2.4	3.1	8.4	8.4	8.2	9.1	42.4	48.4	50.0	11.3	3.1
						P. longimerus								
No. of specimens	81	84	56	167	195	366	265	182	32	126	0	1	41	57
% adults	95.1	100	96.4	48.5	21.6	21.9	17.6	25.3	34.4	73.0	0	100	100	100
% adults that were females	77.9	65.1	20.4	44.5	35.8	40.0	50.0	47.8	27.3	57.6	0	100	68.0	47.0
% ♀$_e$[a]	16.0	32.0	7.0	16.0	6.0	6.0	2.3	1.0	0	0	0	0	4.9	28.4
% ♀$_b$	0	0	1.8	3.5	0	0.5	3.8	1.6	0	0	0	0	0	0
% ♀	61.0	32.4	11.0	1.7	1.0	2.4	0.3	9.3	9.3	42.1	0	100	27.1	24.6
						P. deichmannae								
No. of specimens	13	42	98	172	422	273	213	976	41	41	0	7	2	42
% adults	100	100	100	88.6	71.8	82.8	65.7	17.1	46.4	97.6	0	100	100	100
% adults that were females	46.0	71.4	68.4	58.2	54.5	48.7	24.3	26.0	36.8	75.0	0 0	86.0	100	88.1
% ♀$_e$[a]	0	57.0	52.0	46.5	27.0	25.0	5.0	0.5	0	0	0	0	100	69.0
% ♀$_b$	0	0	2.0	0.5	3.3	1.8	0.5	0.9	0	0	0	0	0	0
% ♀	46.0	14.3	14.4	4.6	8.5	13.1	18.8	3.0	17.1	73.1	0	86.0	0	19.1
Water temp (*C*) at low tide	7.5	12.5	17.0	18.0	21.0	21.5	20.5	24.5	16.5	8.0	0.5	5.5	9.7	14.0

[a] ♀$_e$ = ovigerous females; ♀$_b$ = females with bristles on the brood plates; ♀ = females without bristles on the brood plates.

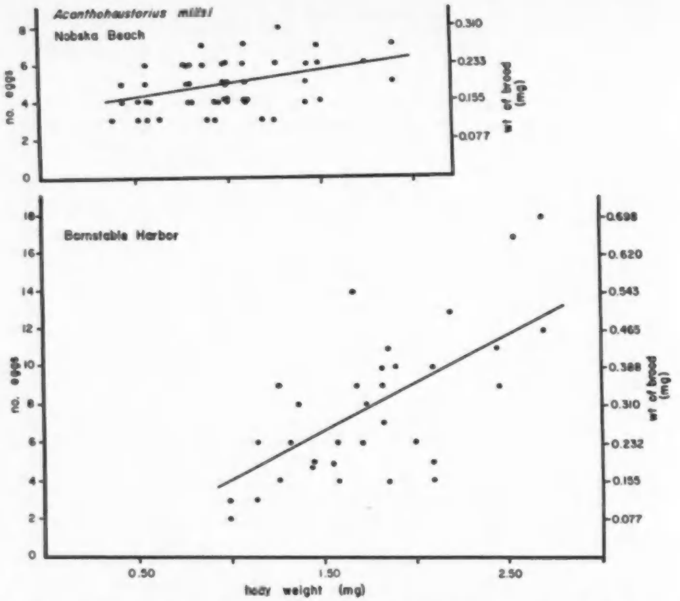

FIG. 5. Relationships of number of eggs per brood and weight of the eggs to dry body weight for *A. millsi* females taken from Nobska Beach and Barnstable Harbor.

weight of the female. The weight of individual eggs (Table 4) did not change with a change in the size of the female or with the different times of the breeding season. The equation for the relationship between the dry weight of the brood of eggs and the body weight of the female was:

$$Y = 0.123 + 0.051\ X$$

which means that for each 1-mg increase in the dry body weight of the female the brood weight increased by 0.051 mg. The weight of the brood related to the female's body weight decreased as the female became larger. The eggs represented 25.0% of the body weight of a 0.50-mg female and only 11.2% of the body weight of a female weighing 2.00 mg.

There was a slight decrease in the brood size through the summer as the larger early breeding females died leaving the late-maturing smaller females to make up the breeding population. Table 4 gives the mean brood size and range of eggs per brood for the entire summer.

The relationship between body length and dry body weight was curvilinear (Fig. 6).

Of the four species of Haustoriidae at Nobska Beach, *A. millsi* was the most abundant in the sands during the fall and early spring and was the only

TABLE 4. Mean numbers of eggs per female, ranges of brood size, and mean weights of single eggs for three species from Nobska Beach and Barnstable Harbor.

Species	Mean no. of eggs	Range of brood size	Mean dry wt (*mg*) of single egg	Total no. of females examined	Total no. of eggs weighed
A. millsi					
Barnstable Harbor	7.8±3.9[a]	4–23	0.038±0.004[a]	34	94
Nobska Beach	4.8±1.3	3–8	0.036±0.005	51	20
P. deichmannae					
Barnstable Harbor	5.7±1.9	2–11	0.023±0.005	46	41
Nobska Beach	3.2±0.8	2–5	0.015±0.005	40	14
P. longimerus					
Barnstable Harbor	8.4±4.0	4–19	0.106±0.010	35	15
Nobska Beach	5.3±1.9	3–11	0.085±0.012	32	69

[a] One standard deviation of the mean.

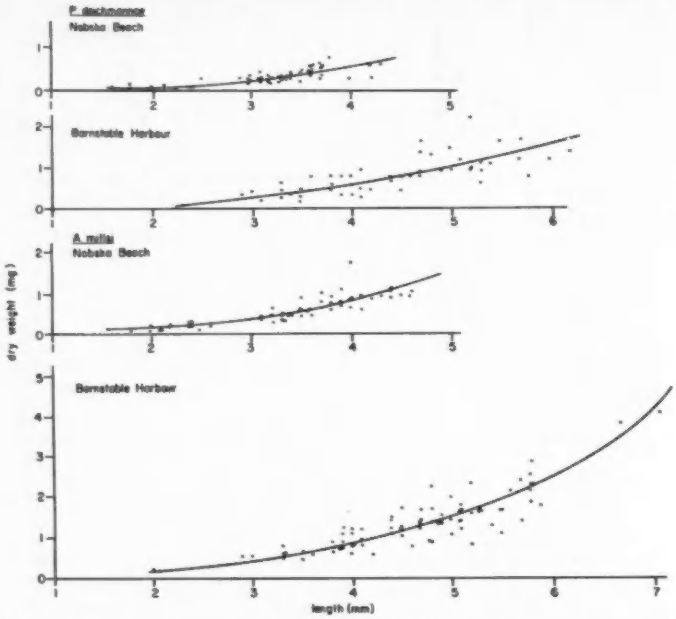

FIG. 6. Relationships between body length and dry body weight for *P. deichmannae* and *A. millsi* in the two study areas.

species present in the February collections. The number of animals per square meter generally increased the further the sample was from the low tide line (Fig. 4). The increase was curvilinear with no indication of a plateau being reached at the ends of the transects. The maximum number of *A. millsi* per square meter was 4605 including both adults and juveniles. The cutoff at the end of each diagram of Fig. 4 does not mean the species was not present beyond that point but that this was the last station sampled. The last stations sampled depended on the weather and tide, therefore the length of the transect varied with different collection dates.

LIFE CYCLE AND FECUNDITY OF *PARAHAUSTORIUS LONGIMERUS*

The life cycle of *P. longimerus* was annual, for mainly juveniles were collected in late summer and early fall (Table 3). The first ovigerous females appeared in late April and early May at the same time and temperature as *A. millsi*. The ovigerous females in the population reached a peak during late May and early June after which time there was a consistent decrease in their number until none were found in the October sample. The number of broods the females produced could not be determined from the data with any accuracy, but due to the low numbers of ovigerous females in the samples taken after the latter part of June, it is unlikely that they had more than one or two broods before dying.

The data suggested that the sex ratio before the reproductive season was close to 1:1 with any deviation tending to favour the females (Table 3). In early June to November the sex ratio was in favour of the males, possibly because of the post-ovigerous female mortality. The high percentage of males in the October collection may be due to the small size of the sample. The number of eggs per brood (Table 4) increased linearly with the dry body weight of the female (Fig. 7.) The regression equation was:

$$Y = 0.64 + 1.39\ X.$$

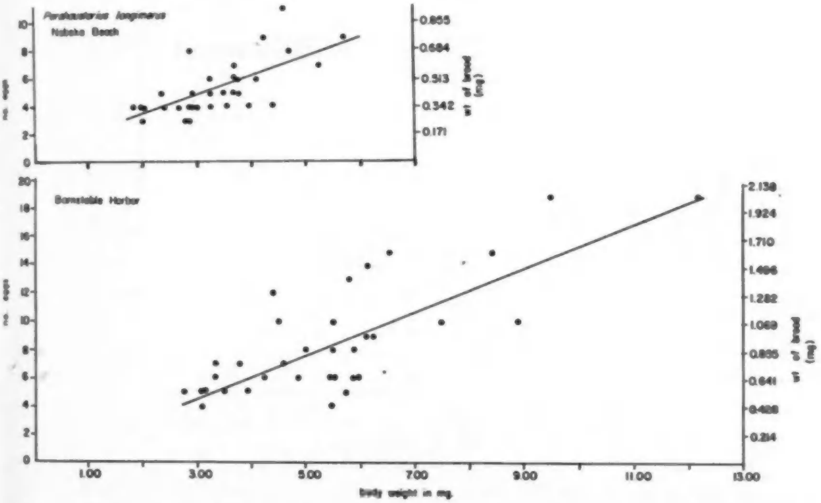

FIG. 7. Relationships of number of eggs per brood and weight of eggs to dry body weight for *P. longimerus* females taken from Nobska Beach and Barnstable Harbor.

The equation for the relationship between the dry body weight and the dry weight of the brood of eggs was:

$$Y = 0.010 + 0.135\ X$$

which means that for each 1-mg increase in the dry body weight the brood weight increased by 0.135 mg. The average weight of eggs within a brood did not vary with size of female or time of year.

The weight of the brood of eggs relative to the body weight of the female did not change appreciably with an increase in the body weight of the female; it ranged from 14.0% for a 2.0-mg female to 13.6% for a 6.0-mg female.

The relationship between the dry body weight and the body length followed a curvilinear regression (Fig. 8).

The number of animals per square meter was lower than for the other species present (Fig. 4, Table 2). There was a slight increase in density with distance from the low tide line, but the increase was much less than for *A. millsi*. The density of *P. longimerus* decreased in the fall and no specimens were found in the February sample.

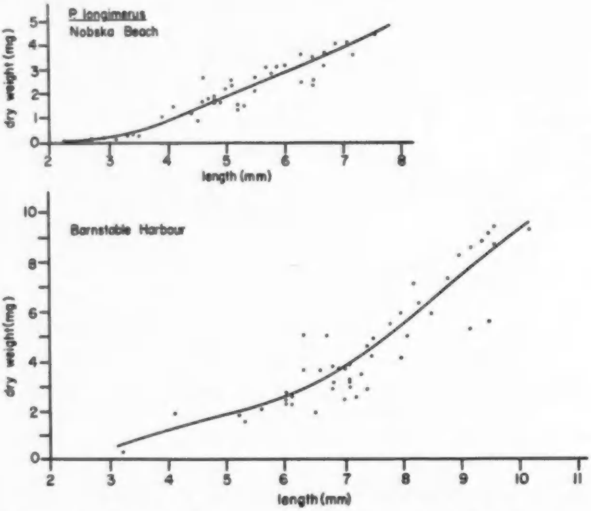

FIG. 8. Relationship between body length and dry body weight for *P. longimerus*.

LIFE CYCLE AND FECUNDITY OF *PROTOHAUSTORIUS DEICHMANNAE*

Protohaustorius deichmannae evidently had a yearly life cycle. Since no specimens were collected in the winter months one cannot say with certainty that a winter generation was not produced in deeper water, but the data tend to disprove the idea, for no juveniles were taken in the April or May collections (Table 3). The fact that only young animals of the previous summer were collected in October also supported the view that the life cycle was annual.

Ovigerous females appeared in early May when the temperature approached 9 C: no ovigerous females were found in the 1966 April collections when the water temperature was 5.5 C. The first juveniles were collected in early June when the water temperature was 17.0 C. Large numbers of ovigerous females were collected until the August 4 collection at which time the numbers dropped. The percentage of ovigerous females in the population followed a pattern similar to that for *Neohaustorius biarticulatus* (Sameoto, 1969), suggesting that the females may have had successive broods of eggs.

Due to the small range in the number of eggs per brood (Table 4) it was not possible to calculate an accurate relationship between the body weight and the brood size (Fig. 9).

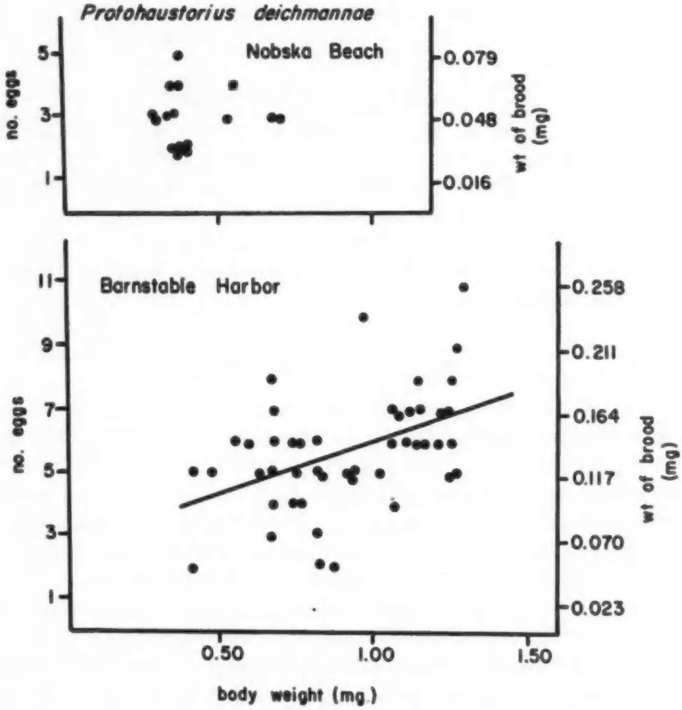

FIG. 9. Relationships of number of eggs per brood and weight of eggs to dry body weight for *P. deichmannae* taken from Nobska Beach and Barnstable Harbor.

The relationship between the dry body weight and the body length was slightly curvilinear (Fig. 6).

The first animals appeared in the sands at the beginning of April in 1966, but large numbers were not found in 1965 until the middle of June when the water temperature was near 17 C. The number of animals per square meter increased in an exponential manner as the sampling proceeded seaward during all the collection periods (Fig. 4). The mean density of animals generally increased until August; in October there was a marked decrease (Table 2).

BATHYPOREIA SP.

Associated with the three above species were two species of *Bathyporeia*. These appeared in the samples in April in 1966 and decreased in number in the fall. The distribution of *Bathyporeia* along a transect was very similar to that of *P. deichmannae*, as it generally followed an exponential rate of increase with successive samples seaward. But in two of the collections a sudden drop in the density of *Bathyporeia* occurred at about the 35-m station. This may indicate that the species was limited to the inshore waters in the summer.

OTHER MACROFAUNA COLLECTED WITH HAUSTORIIDAE

Very few other species were collected from the two transects. In May, *Scolopolos armiger* was abundant along transect 1. During April and early May, *Neohaustorius schmitzi* Bousfield was commonly caught in low numbers, but disappeared when the other species of Haustoriidea became abundant in late May. Only two specimens of *Chiridotea coeca* were collected, these in June. *Paraphoxus epistomus* (Shoemaker) was occasionally found in the samples throughout the summer.

BARNSTABLE HARBOR SPECIES

The sand bar at Barnstable Harbor contained populations of *A. millsi*, *P. longimerus*, and *P. deichmannae*, but did not have a population of *Bathyporeia*.

The density of each species varied with different locations on the bar, but the species were found over the entire bar with the exception of the coarser sands. Due to the instability of the environment and the large fluctuations in the number of specimens collected from period to period, it was difficult to obtain satisfactory data on the distribution of the species over the bar, or on fluctuations in the species densities with time, although from Table 5 it can be seen the numbers dropped considerably during the late fall. No haustoriids were found at stations on top of a series of small high bars of very coarse sand (>2.00 mm) and few were found in the small channels between the large main bar and the small ones adjacent to it. *Protohaustorius deichmannae* was most abundant in the southern half of the bar where the sands were not well sorted ($DD\phi = 0.7$). The other two species appeared to be more abundant in the northern half of the bar, in the sands which were well sorted ($DD\phi = 0.3$). The northern half was the first region of the bar to be exposed at low tide and was drained of most of its interstitial water. Here the animals were almost entirely found in the troughs of the sand ripples that remained water-saturated.

No animals were found in a shallow submerged flat between two arms of the bar. This sand was black 1 or 2 cm below the surface. The density of haustoriid dropped suddenly as the samples reached the water line at the edge of the sand bar. The reason for this is not known, but it may be related to the oxygen conditions in the sand. The submerged sands appeared to be darker than those exposed at low tide. Correlation coefficients were calculated between the numbers of *P. deichmannae* and *A. millsi* and the various sand sizes in the percentile fractions of the sand. The only significant correlations (r) found

TABLE 5. Mean numbers of three species per m^2 at Barnstable Harbor on various dates, 1965 and 1966.

Date	*P. deichmannae*	*A. millsi*	*P. longimerus*	No. of samples
1965				
May 27	1425±195[a]	645±150	5±5	6
June 8	825±195	480±525	114±226	11
June 22	990±330	555±225	30±54	13
July 7	3360±1350	2250±900	30±95	9
Aug. 4	1410±135	630±240	15±30	12
Aug. 19	1755±60	300±45	15±15	12
Oct. 2	3345±225	1590±540	0	11
Nov. 19[b]	495	120	15	10
1966				
Apr. 4[b]	330	135	30	10
May 5[b]	450	750	30	10

[a] One standard deviation of the mean.
[b] Means of 10 samples grouped together.

were between the coarser sands in the 100, 90, and 80 percentile sand fractions and *P. deichmannae* ($r = -.51, -.51, -.49$). This suggested that this species avoided areas with small amounts of coarse sand even though the preferred finer sands were also present. The coarser these portions of the sand were, the fewer amphipods there were present. The possibility of this correlation being fortuitous cannot be overlooked. It might have been related to the tidal current which removed much of the fine sand and also may have removed the animals at the same time, depositing the two in different locations on the bar.

No significant correlation was found between the distribution of any of the three species and the per cent organic matter of the sand.

LIFE CYCLE AND FECUNDITY OF *ACANTHOHAUSTORIUS MILLSI*

Acanthohaustorius millsi started to breed in early April when the water temperature was 5.5 C. The number of ovigerous females reached a peak in the early part of June and declined steadily throughout the summer (Table 6). This suggested that the females may have had only one brood as at Nobska Beach.

The mean number of eggs per brood and the range of the brood size are given in Table 4. The relationship between the dry weight of the female and the number of eggs in the brood was linear (Fig. 5) with the regression equation:

$$Y = -2.47 + 5.85\ X.$$

The regression did not deviate significant by from linearity. The negative value for the intercept has no biological significance, for no values existed below 0.75 mg. The relationship between the brood weight and the body weight was a linear regression with the equation:

$$Y = -0.031 + 0.188\ X.$$

TABLE 6. Percentages of collections of two species of Haustoriidae from Barnstable Harbor on various dates, 1965 and 1966, that were adults, of the adults that were females, and of the collections that were females in various stages of maturation.

	1965							1966	
	May 25	June 8	July 7	Aug. 4	Aug. 19	Oct. 2	Nov. 19	Apr. 4	May 5
				A. millsi					
No. of specimens	368	227	451	169	61	324	119	92	62
% adults	96.0	100	57.4	48.0	59.1	67.3	90.0	100	100
% adults that were female	47.6	36.2	37.5	34.6	30.6	43.1	64.2	42.4	41.9
% ♀$_e$[a]	33.4	17.6	11.5	4.1	0	4.3	5.0	1.0	22.6
% ♀$_b$	1.6	11.4	2.9	0	0	0.6	0	0	3.2
% ♀	6.5	7.0	7.1	12.4	13.1	24.1	52.1	41.3	16.1
				P. deichmannae					
No. of specimens	425	190	692	378	348	689	655	221	374
% adults	86.9	100	49.0	82.3	91.7	61.0	96.5	100	100
% adults that were females	53.4	52.2	42.0	43.1	43.3	43.1	61.4	46.7	41.9
% ♀$_e$[a]	38.8	48.9	14.3	24.6	18.7	12.6	3.3	2.7	33.4
% ♀$_b$	1.9	1.0	0.7	2.3	3.2	0	0	0	1.3
% ♀	5.4	2.3	5.5	10.6	17.8	13.6	55.9	44.0	7.2
Water temp (*C*) at low tide	19.5	22.0	20.0	19.5	25.0	15.5	4.5	5.5	13.5

[a] ♀$_e$ = ovigerous females; ♀$_b$ = females with bristles on the brood plates; ♀ = females without bristles on the brood plates.

This equation is significantly different ($F = 9.68$, df $= 2.81$; $P < .01$) than that for *A. millsi* found at Nobska Beach. The brood weight relative to the body weight of the female decreased from 22.9% of a female weighing 0.75 mg to 20.0% of a female weighing 0.50 mg. The relationship between the body length and the body weight was curvilinear as seen in Fig. 6.

LIFE CYCLE AND FECUNDITY OF *PARAHAUSTORIUS LONGIMERUS*

Parahaustorius longimerus had the lowest density of the three species, being absent from a majority of the samples. Therefore the number of specimens was too low to draw any conclusions on density fluctuations through the year. The first ovigerous females were collected on May 5 and the last on August 4. Due to the very low numbers of animals collected it is not possible to say with certainty that these two dates represent the start and end of the breeding season. There was an indication that the number of ovigerous females reached a peak in the latter part of May, then declined, as was found at Nobska Beach.

The sex ratio appeared to be 1:1 at the start of the breeding season, but due to the small samples this is in doubt. The mean number of eggs per brood and the size range of the broods is given in Table 4. The relationship between the dry body weight of a female and the size of the brood followed a linear regression (Fig. 7) with the equation:

$$Y = 0.35 + 1.46\,X.$$

The relation between the body weight and the brood weight was also linear with the equation:

$$Y = 0.036 + 0.156\,X.$$

There was no significant difference between these regressions and those for *P. longimerus* found at Nobska Beach. There was no appreciable difference in the relative weights of the brood and the female's body weight with increased female size. The range was 16.8% for a 3.0-mg female to 15.9% for 12.0-mg female.

The relationship between the body weight and the body length was curvilinear as shown in Fig. 8.

LIFE CYCLE AND FECUNDITY OF *PROTOHAUSTORIUS DEICHMANNAE*

Ovigerous females of *P. deichmannae* were present in the April 4 collection when the water temperature was 5.5 C; the population had the largest percentage of ovigerous females in late May and early June (Table 6). The percentage of ovigerous females remained high relative to *A. millsi* until the November samples. This suggested that the females produced more than one brood a year, or that the early new generation matured fast enough to breed in the late summer and fall. No juveniles were found in the April collections, therefore it is unlikely that a winter generation occurred. If there had been one, the young would have been apparent in the spring collection, since the rate of growth of amphipods is very slow in the winter (Mills, 1967; Sprules, 1967; Sameoto, 1969).

The mean number of eggs per brood and the range of the number of eggs per brood are given in Table 4. The relation between the number of eggs per brood and the dry body weight of the females was linear (Fig. 9). The regression equation was:

$$Y = 2.69 + 3.36\,X.$$

The relationship between the dry weight of the brood and the dry body weight of the female was expressed in the equation:

$$Y = 0.062 + 0.078\,X.$$

This means that with each 1-mg increase in the dry body weight the dry brood weight increased by 0.078 mg. The weight of the brood relative to the weight of the female decreased as the female became larger. The broods represented 20.2% of the body weight of a 0.5-mg female and only 11.9% of the weight of a 1.5-mg female.

The relationship between the body length and the body weight was curvilinear as seen in Fig. 6.

The sex ratio at all times of the year appeared to be close to 1:1, with a slight increase in the number of males in the summer possibly due to a higher rate of post-reproductive mortality in the females.

BODY SIZES OF SPECIES IN THE TWO AREAS

The adult populations of the three species of Haustoriidae at Barnstable Harbor grew larger than they did at Nobska Beach (Fig. 5, 7, and Tables 7, 8). The Barnstable Harbor animals followed similar body length and weight curves as did the animals at Nobska Beach except that they went further in growth. Random samples of adults of each species from the two areas collected the same day were measured. The two sexes of each species at Barnstable Harbor were larger with two exceptions. ***Protohaustorius deichmannae*** males collected May 22 and *A.,millsi* females collected July 7 were not significantly different in body size at the two locations.

The largest and the smallest ovigerous females were measured in samples from Nobska Beach and Barnstable Harbor after each collection. Table 8 shows that the smallest ovigerous females at Barnstable Harbor were much larger than the smallest found at Nobska Beach. The largest ovigerous females at Barnstable Harbor were much larger than the largest found at Nobska Beach. Ovigerous females were selected randomly from the May 22 samples and measured to find the difference in the mean body lengths between the two populations (Table 8). It was found that the ovigerous females at Barnstable Harbor were about 20% larger on the average than those at Nobska Beach.

Table 7. Comparison of mean body lengths (mm) of males and females of three species found at Nobska Beach and Barnstable Harbor during 1965.

	Males			Females		
	Nobska	Barnstable	Value of *t* and df	Nobska	Barnstable	Value of *t* and df
			A. millsi			
May 6	3.66±0.37[a]			4.16±0.44[a]		
May 22	3.53±0.35	4.27±0.81[a]	3.80**[b]; 54	4.15±0.39	5.25±0.77	5.84**; 45
June 22	3.36±0.20	4.49±0.75	3.90**; 35	3.99±0.33	5.40±0.67	8.41**; 40
July 7	3.27±0.26	4.30±0.66	2.92*[b]; 17	4.71±0.48	4.80±0.58	Not significant; 21
			P. deichmannae			
May 22	3.33±0.27	3.85±0.65	Not significant; 19	3.71±0.54	4.95±0.66	6.75**; 48
June 22	3.20±0.19			3.68±0.26	5.15±0.62	8.32**; 32
July 7	2.99±0.80	4.16±0.72	2.58*; 11	3.56±0.34	4.97±0.58	6.64**; 18
			P. longimerus			
May 6	5.33±0.76			5.97±1.08		
May 22	4.89±0.53	6.24±0.88	5.01**; 25	6.24±1.19	7.79±1.23	4.79**; 56
June 22	4.80±0.55	7.10±0.97	7.82**; 33	6.06±0.88	8.51±1.27	4.87**; 18
July 7	4.61±0.62	6.20±0.61	4.19**; 18	6.34±0.96	8.77±0.76	6.21**; 24

[a] One standard deviation of the mean.
[b] *Significant at $P = 0.05$; **significant at $P = 0.01$.

TABLE 8. Comparison of the body lengths (mm) of ovigerous females of three species found at Barnstable Harbor and Nobska Beach during 1965.

	Ranges		Means			
	Nobska	Barnstable	Nobska	Barnstable	% difference	Value of *t* and df
			A. millsi			
May 6	3.51–5.11					
May 22	3.64–5.02	4.30–7.00	4.50±0.8[a]	5.30±0.5[a]	22	7.18*[b]; 83
June 22	3.47–4.62	4.49–7.12				
July 7	4.00–5.50	4.70–5.51				
			P. deichmannae			
May 22	2.97–4.62	3.85–6.15	3.81±0.6	4.70±0.7	20	6.43*; 63
June 22	3.43–4.31	4.04–6.17				
July 7	3.10–4.20	4.50–5.70				
			P. longimerus			
May 6	4.44–8.49					
May 22	4.75–8.44	6.00–12.10	6.50±1.0	7.90±1.2	18	5.40*; 65
June 22	4.97–7.64	6.90–10.18				
July 7	5.30–8.40	7.30–9.50				

[a] One standard deviation of the mean.
[b] *Significant at $P = 0.01$.

GUT CONTENTS

The gut contents of 10 specimens of each species from Nobska Beach and Barnstable Harbor were examined. The only recognizable items *P. longimerus* contained were diatoms and small sand grains, both of which were abundant. No animals or animal parts were seen. The most common material was greenish-brown amorphous organic matter.

Guts of *A. millsi* contained similar material to that of *P. longimerus*, the only difference being the smaller size of the sand particles. Again no animal matter was found.

Gut contents of *P. deichmannae* were similar to the other two species, but appeared to be less well sorted. The gut contained diatoms, sand, and the same greenish-brown material. Specimens of this species from Barnstable Harbor contained, in addition to the above-mentioned material, the remains of suctorian ciliates and small Crustacea.

Observations using an upside-down microscope showed that all three species fed while burrowing through the sand. The large amount of sand found in the guts of the species supported these observations.

DISCUSSION

The differences in the densities of the two species of Haustoriinae and *Bathyporeia* spp. on the two transects at Nobska Beach were probably due to the different sand conditions on the transects. The sands of transect two were generally coarser and not as well sorted as those of transect one. In addition, no sand bar was built up during the summer on the second transect. These sand conditions may have been the result of the greater wave action over the central part of the beach. The *Bathyporeia* species were the only ones that showed an increase in density at transect two. The increase in adult density of all the species with the increase in water temperature suggests there was a migration into the intertidal zone in the summer. A migration back to deeper water occurred in the fall when the temperature decreased.

The distribution of the Haustoriidae at Barnstable Harbor was partially related to the grain size of the sand bar. This observation was supported by experimental evidence (Sameoto, unpublished data). The coarser the sand the lower the number of amphipods found in it, with none being found in the coarsest sands (3.0–1.0 mm in diameter). Few amphipods were found in sands that were not exposed to the air at low tide. This may have been because of the low oxygen conditions in these sands.

The first ovigerous females of *P. deichmannae* and *A. millsi* at Nobska Beach were collected when the water temperature was 7.5 C, whereas the first ovigerous females of these species at Barnstable Harbor appeared at 5.5 C. Due to the low number of specimens taken at Nobska Beach this temperature difference probably did not represent different breeding temperatures for these populations.

All three species started to reproduce in the early spring and reproduction was almost finished by the end of August. This was opposite to the reproduction cycle of *P. longimerus* and an *Acanthohaustorius* sp. from Sapelo Island, Georgia (Croker, 1967a). Here the reproductive season started in August and ended in May and June. It would be interesting to know if this difference is a response to the different water temperatures (the mean annual temperature at Woods Hole is about 9 C and at South Carolina 20 C), or a genetic difference.

The mature individuals of each species at Barnstable Harbor were significantly larger than those at Nobska Beach, and this difference in body size resulted in the Barnstable species having larger brood sizes. The smallest ovigerous females collected at Barnstable Harbor were larger than the smallest ovigerous females taken at Nobska Beach. This indicated that the Barnstable Harbor amphipods were larger when they matured. How this increase in growth occurred, whether by increased growth at each instar or by additional instars is uncertain.

The factors found to be different between the two environments were the per cent organic matter of the sand and sand temperature at low tide and the water temperature at high tide. Barnstable Harbor sand had a higher organic content than that at Nobska Beach, which may have reflected a greater amount of food available, since the animals fed on interstitial detritus and diatoms (Croker, 1967a). Kinne (1959) stated that a lack of green plant food caused inhibition of growth, increased mortality, and a decrease in egg number in *Gammarus duebeni* Liljeborg. Also, an insufficient quantity of food caused retardation of growth in this species. A difference in the quality and quantity of the food may have been a factor for the difference in size between the Barnstable Harbor and Nobska Beach populations.

Before any definitive statements are made concerning the amount of food in the two areas one will have to determine what constitutes food for these species. Gut content analysis showed that unrecognizable detritus and diatoms were ingested, but what was digested from this is not known. In addition, the role of bacteria in the food will have to be examined before any comparison of the relative amounts of food in the two regions can be made.

The other environmental difference between the two areas was the sand temperature at low tide and the water temperature at high tide. Barnstable Harbor was exposed to the air for up to 2 hr during low tide, at which time it was heated by the sun to temperatures up to 26 C. Ayers (1966) gave an analysis of the heat budget of Barnstable Harbor and showed that temperatures of the emerged sand bars reached 35 C 1 hr after low tide. Therefore, the species at Barnstable Harbor were likely exposed to higher temperatures for short periods of time than those at Nobska Beach. The yearly mean water temperature for Barnstable Harbor in 1965 was 8.2 ± 5.6 C and for Nobska Beach it was 10.3 ± 7.8 C. It is possible that this temperature difference was part of the cause for the larger size of the Barnstable animals. It is a common phenomenon for invertebrate species to become larger with decreasing environmental temperatures (Mann, 1947; Gunter, 1957; Ray, 1960, McLaren, 1963; and Lindsay, 1966).

All three species showed similar relative differences in size at the two locations, which suggests the influence of an ecological factor. However, other than environmental factors may be involved. Differences in the mean egg weight were found with the two populations of *P. deichmannae*, but not with the other two species. Also the regression lines for body weight with the egg number were significantly different for the *A. millsi* populations ($F = 9.68$; $df = 2.81$; $P < .01$). In addition the proportional relationship between the body weight and the weight of the brood of eggs changed as the *A. millsi* females at Nobska Beach became larger, but did not change with increasing body size for *A. millsi* females at Barnstable Harbor. Whether this is due to an ecological or genetic factor is unknown.

The body length ratio between *A. millsi* and *P. longimerus* was 1.0:1.5 at Nobska Beach and 1.0:1.7 at Barnstable Harbor. Similar body ratios have been found for other species of Haustoriidae (Croker, 1967a, b; and Sameoto, 1969). The larger animals have larger mouth parts and therefore are able to ingest a larger size range of food particles. All the species in this study had similar food materials in their guts, the only noticeable difference being the larger sized sand grains in *P. longimerus*.

Observations of the animals in aquaria by myself and in the field by Croker (1967a, b), suggested that *P. deichmannae* mainly occupied the top 1 or 2 cm of sand, whereas *A. millsi* and *P. longimerus* inhabited the deeper sands. Since the three species fed on similar types of food the vertical separation of *P. deichmannae* from the other two species would lessen competition for food between *P. deichmannae* and the other haustoriids. Differences in size of the mouth parts and body size have been cited as a possible way of reducing competition for food in coexisting species that feed on the same type of food by allowing them to feed on different size ranges of food (Hutchinson, 1959; and Croker, 1967b).

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REFERENCES

AYERS, J. C. 1966. A late summer heat budget of Barnstable Harbor, Massachusetts. Limnol. Oceanog. (Suppl.) 10: R 9–14.

BRAFIELD, A. E. 1964. The oxygen content of interstitial water in sandy shores. J. Animal Ecol. 33: 97–116.

BOUSFIELD, E. L. 1962. New haustoriid amphipods from the Canadian Atlantic Region. Natl. Museum Canada Bull. 183: 42–62.

1965. Haustoriidae of New England (Crustacea: Amphipoda). Proc. U.S. Natl. Museum 117: 159–240.

CROKER, R. A. 1967a. Niche diversity in five species of intertidal amphipods (Crustacea: Haustoriidae). Ecol. Monographs 37: 173–200.

1967b. Niche specificity of *Neohaustorius schmitz* and *Haustorius* sp. (Crustacea: Amphipoda) in North Carolina. Ecology 48: 971–975.

GUNTER, G. 1957. Temperature, p. 158–184. *In* J. W. Hedgpeth [ed.] Treatise on marine ecology and paleoecology. Geol. Soc. Am. Mem. 67, Vol. 1.

HUTCHINSON, G. E. 1959. Homage to Santa Rosalia *or* why are there so many kinds of animals? Am. Naturalist 93: 145–159.

KINNE, O. 1959. Ecological data on the amphipod *Gammarus duebeni*. A monograph Veröff. Inst. Meeresforsch. Bremerh. 6: 177–202.

LINDSEY, C. C. 1966. Body size of poikilotherm vertebrates at different latitudes. Evolution 20: 456–465.

MANN, G. 1947. Biologia de la Antarctica Suramericana. Inst. Geography Univ. Chile. Publ. 2. p. 346.

MCLAREN, I. A. 1963. Effects of temperature on growth of zooplankton and the adaptive value of vertical migration. J. Fish. Res. Bd. Canada 20: 685–727.

MILLS, E. L. 1967. The biology of an ampeliscid amphipod crustacean sibling species pair. J. Fish. Res. Bd. Canada 24: 305–355.

RAY, C. 1960. The application of Bergmann's and Allen's rules to poikilotherms. J. Morphol. 106: 85–108.

SAMEOTO, D. D. 1969. Comparative ecology of intertidal sand-burrowing amphipods (Crustacea: Haustoriidae). J. Fish. Res. Bd. Canada 26: 361–388.

SPRULES, W. G. 1967. The life cycle of *Crangonyx richmondensis laurentianus* Bousfield (Crustacea: Amphipoda). Canadian J. Zool. 45: 877–884.

Some Chemical Factors Affecting the Early Developmental Stages of *Euchaeta japonica* (Crustacea: Copepoda: Calanoida) in the Laboratory

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LEWIS, A. G., AND A. RAMNARINE. 1969. Some chemical factors affecting the early developmental stages of *Euchaeta japonica* (Crustacea: Copepoda: Calanoida) in the laboratory. J. Fish. Res. Bd. Canada 26: 1347–1362.

Survival of the egg and naupliar stages of *Euchaeta japonica* Marukawa reared in the laboratory was increased by addition of zinc, cobalt, and a chelating agent (Na_2EDTA) to sea water from the natural environment. The increase, however, occurred during varying times of the year. The benefits of each of these three substances as well as the periodicity are discussed.

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INTRODUCTION

IT IS UNDERSTOOD that phytoplankton require dissolved organic and inorganic materials for growth (e.g., Lewin, 1962) and that at least some aquatic animals can take up and may require dissolved organics (Shiraishi and Provasoli, 1959; Stephens and Schinske, 1961). These are in addition to the particulate materials used as food and the various environmental requirements such as acceptable levels of temperature and salinity. The present study forms part of a general examination of the requirements of the life-history stages of zooplanktonic organisms but is itself concerned with the requirements for dissolved materials exhibited by the egg and naupliar stages of the planktonic copepod *Euchaeta japonica* Marukawa.

Most of the studies on specific requirements of planktonic organisms have been done on phytoplankton and have been carried out in the laboratory. Laboratory studies of this type necessitate somewhat artificial conditions and the problems of interpretation that go along with them. This appears essential, however, as the controlled conditions important for the determination of the requirements are difficult if not impossible to establish in the field. Field work can, on the other hand, suggest the need for certain lines of laboratory research on the requirements of marine organisms by demonstrating faunal and floral differences in space (i.e., water types) and in short periods of time. Thus, as was pointed out by Bary (1963), there can be biological differences between different bodies of water.

If there are demonstrable biological differences between bodies of water it implies an association of the organism with one or more types of water. This further suggests that these water bodies possess certain characteristics

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that are beneficial to some organisms but not to others, as indicated by the laboratory studies of Wilson (1951), Wilson and Armstrong (1952), and others (see Provasoli, 1963).

If the concept of water body–plankton associations is examined with the laboratory-obtained knowledge that certain organisms require dissolved nutrients, it indicates that the absolute or relative quantity of these substances could, at critical levels, be a limiting factor. What is needed to explore this possibility is to determine the nature of the required dissolved materials as well as the amounts that are necessary. In organisms that have a multistage life cycle it is also necessary to determine what stage or stages of development are most susceptible to deviations from the optimum level of the dissolved substances. Further, it would be necessary to determine whether or not the different life-history stages require different types or amounts of the dissolved materials. To complete the laboratory phase of the research it would ultimately be necessary not only to determine the mechanism used to obtain and control the required amounts of the dissolved substances but also the role that they play in the physiology of the organism. One of the applications of this knowledge is in the explanation of the distribution and annual survival of the organism. A second is the indication of possible reasons for the association of the organism with a particular water body or at least the general conditions under which the organism is best maintained.

To best explore the water body–plankton associations it is advisable to utilize an organism, or series of organisms, that is restricted to one or a few water bodies. To determine the requirements of the organisms in the laboratory it is first necessary to determine the effects of various types of laboratory equipment, techniques, and food media on the survival of the life-history stages. This process is most readily carried out by using an organism that is not only restricted to certain water bodies but is readily obtained and produces eggs throughout the year. The calanoid copepod *E. japonica* was found to best fit the requirements for a test organism.

Euchaeta japonica has a wide distribution in the North Pacific (Davis, 1949). In the Strait of Georgia, between Vancouver island and the mainland, the ovigerous female and early developmental stages are most frequently found in water which Waldichuk (1957) calls Strait of Georgia deep water, suggesting an association of the organism with water of a particular origin. The life history of the species includes an egg stage, six naupliar stages (Fig. 1), and six copepodite stages (Campbell, 1934). The adult female is relatively large (5.4–8.0 mm, Davis, 1949; 6.3–6.5 mm, Fulton, MS, 1968) and produces a cluster of 8–24 deep-blue eggs which remains attached to the genital segment for at least part of the embryonic development. The egg and first two naupliar stages utilize stored reserves for development whereas the third through sixth nauplius stages rely, at least to some extent, on phytoplankton (A.S. Pandyan, unpublished data). All six copepodite stages are capable of a carnivorous diet but have been found to feed on large members of the phytoplankton to some extent (A. S. Pandyan, unpublished data.)

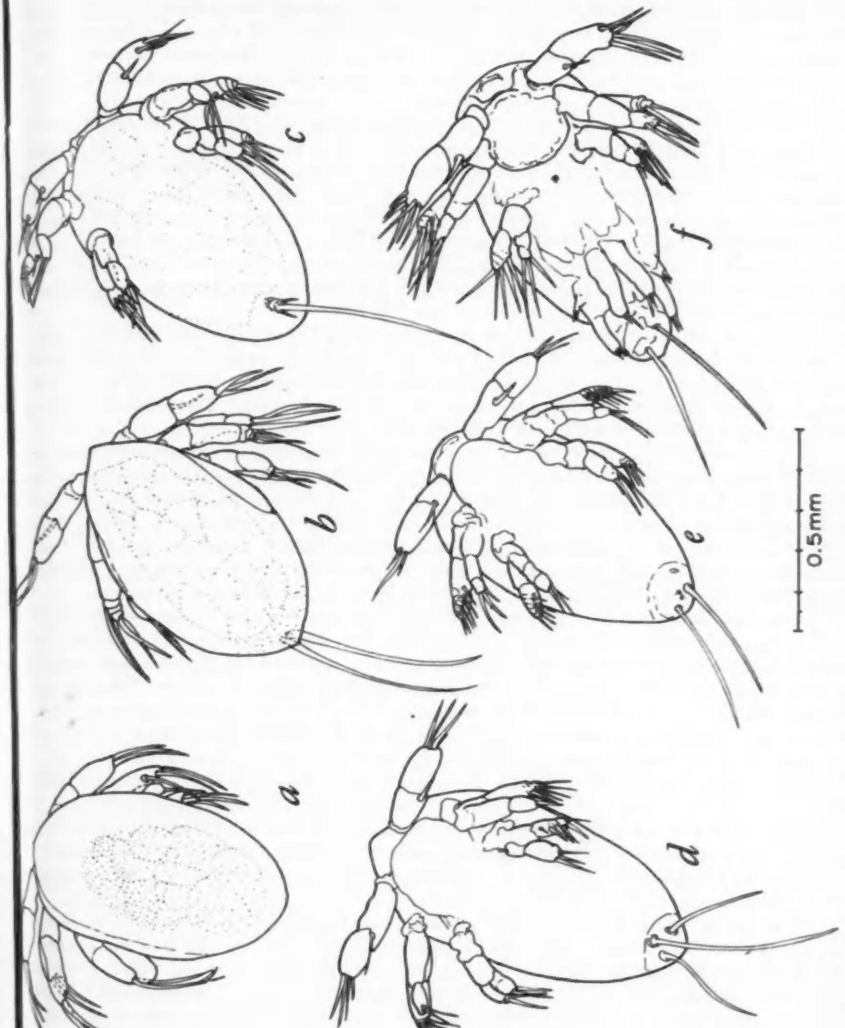

FIG. 1. Naupliar stages of *Euchaeta japonica* Marukawa. *a*, First nauplius, dorsal view; *b*, second nauplius, dorsal view; *c–f*, third through sixth nauplii, ventral views.

MATERIALS AND METHODS

Monthly collections of animals and sea water were made between January 1966 and July 1968 at a station (IOUBC/GS-1) located at 49°17′06″N, 123°50′00″W, between Vancouver Island and the mainland of British Columbia. Sea water was collected from an approximate depth of 350 m by means of a 96-liter sampler, and ovigerous females, as well as free egg clusters, were collected with a 1-m ring net (no. 3 netting) hauled vertically from approximately 400 m to the surface.

The sea water was either filtered immediately or temporarily stored, in 5-gal polyethylene carboys, for later filtration. Filtration was done with a filter $0.45\ \mu$ in mean pore size (Millipore type HA; Gelman type GA-6; Sartorius type MF 50). The filtered sea water was then stored at 8 C in 5-gal polyethylene carboys that had been rinsed twice with distilled water and once with filtered sea water. No attempt was made to keep the sea water bacteria-free.

The zooplankton were sorted in white polyethylene basins (34 × 29 × 14 cm) and ovigerous specimens of *E. japonica* as well as free egg clusters removed with a large bore dropping pipet (6 mm ID). Up to 40 isolated animals and egg clusters were placed in each of several 2-liter wide-mouthed thermos flasks (Dewar "Isotherm" Vacuum Flask) filled with coarsely filtered (no. 25 plankton netting) 350-m sea water. To determine if any of the egg clusters were damaged in handling, they and the ovigerous females were transferred to large finger bowls (19 cm in diameter) in the laboratory and left overnight at 10 C. The egg clusters were then sorted and only those containing eggs that were of the same dark blue color as unfertilized eggs in the oviduct were retained.

A temperature of 10 C was maintained for the storage of the eggs and during the tests. Although the water at 350 m at IOUBC/GS-1 is approximately 8 C, local refrigeration authorities felt that 10 C was the minimum temperature that the equipment would maintain over long periods of time. Water of 10 C occurs in local inlets at a depth where *E. japonica* is found so that the temperature does not appear to be extreme.

The laboratory equipment used in the experiments was tested to determine its effect on the life-history stages. Not only was the effect of the material used in the equipment in question but, when a number of similar pieces of equipment was used, the possible effect of the individual pieces in the series. The problem is the variation in production line techniques found in plastics (Autian, 1964) as well as in antibiotics (Marshall and Orr, 1958) not only between two manufacturers but also, in batch products, by a given producer. This, combined with the variabliity in test organisms (e.g., Bernhard et al., 1966) made testing of all equipment mandatory.

The usual experimental procedure was to randomly select one egg cluster and put it in a 1-liter polypropylene Erlenmeyer flask containing 600 ml of filtered sea water enriched with the chemicals to be tested. Polypropylene flasks (Nalgene flask no. 10-041-10) were used because of their translucent nature and ease of handling. The percentages of survival of the egg and naupliar stages in these flasks were found to be comparable or slightly better than those in new or used (for phytoplankton cultures only), acid-washed, Pyrex Erlenmeyer flasks. Eight flasks were normally used for each test although as few as 4 and as many as 16 were used.

The flasks were kept in two New Brunswick Psychrotherm Incubator Shakers (Model G-27), each with a rotary table that cycles approximately 45 times per minute and which is capable of holding 16 1-liter flasks. Movement of the flasks was used to provide continuous circulation of the sea water and entrained materials and, as shown by ZoBell and Anderson (1936), to inhibit the multiplication of bacteria. To reduce the amount of light reaching the organisms, the window in each of the shakers was covered.

During the period of approximately 4 weeks necessary for the development from the egg through the sixth nauplius the water was changed once every 3 days. At this time the condition of the eggs and nauplii was noted and the dead organisms removed. Food was added, when the water was changed, if any of the organisms were in the feeding stages of development. Aliquots of bacteria-free cultures of *Phaeodactylum tricornutum* and *Dunaliella* sp. were washed (by centrifugation and resuspension) with filtered sea water to remove the culture medium. Enough resuspended cells were added to the flasks to provide a 2:1 ratio of *Phaeodactylum*:*Dunaliella* and a final concentration of 6000 cells per ml.

ENRICHMENT MEDIA

The enrichment medium that was first tested (CEM1, Table 1) is similar to the "ES" seawater enrichment medium of L. Provasoli (unpublished data) for culturing phytoplankton and seaweeds although it contains less iron and six additional vitamins (Lewis, 1967). This medium was found to reduce the mortality of the egg and naupliar stages when added to 350-m filtered sea water collected during certain months but not when added to water collected during others. To determine if the benefit could be enhanced various chemicals were added. To determine the

TABLE 1. Compositions of enrichment media. All concentrations used in the tests were 2% unless otherwise indicated.

CEM1	
Distilled water	100 ml
$NaNO_3$	350 mg
Na_2 glycerophosphate	50 mg
P2 metals (see below)	25 ml
Vitamin B_{12}	10 μg
Thiamine	0.5 mg
Biotin	5 μg
Niacin	888 μg
dl pantothenol	888 μg
Riboflavin	444 μg
Ascorbic acid	1 mg
Pyridoxine HCl	888 μg
Tris buffer	500 mg
P2 metals (for 100 ml use):	
H_3BO_3	114 mg
$FeCl_3 \cdot 6H_2O$	4.9 mg
$MnSO_4 \cdot 4H_2O$	16.4 mg
$ZnSO_4 \cdot 7H_2O$	2.2 mg
$CoSO_4 \cdot 7H_2O$	0.48 mg
Na_2EDTA	100 mg
CEM1 + vitamin E	
CEM1 + 62.5 μg of di alpha tocopherol	
CEM1 + nicotinamide	
CEM1 + 888 μg of nicotinamide	
CEM1A	
CEM1 without P2 metals but with full amount of Na_2EDTA	
CEM1B	
CEM1 without group 1 vitamins (biotin, B_{12})	
CEM1C	
CEM1 without group 2 vitamins (niacin, riboflavin, ascorbic acid, pantothenic acid)	
CEM1D	
CEM1 without group 3 vitamins (thiamine, B_6, choline chloride)	
CEM1E	
CEM1 without group 1 metals (Mn, Fe) but with full amount of Na_2EDTA	
CEM1F	
CEM1 without group 2 metals (Zn, Co, Bo (included in metal group for convenience)) but with full amount of Na_2EDTA	
CEM1G	
CEM1 without zinc but with full amount of Na_2EDTA	
CEM1H	
CEM1 without cobalt but with full amount of Na_2EDTA.	
CEM1I	
CEM1 without boron but with full amount of Na_2EDTA	
CEM2	
CEM1 + 29.7 mg of glutathione	
CEM3	
Distilled water	2000 ml
Valine	120 mg
Leucine	134 mg
Phenylalanine	169 mg
Tryptophane	209 mg
Histidine	159 mg
Arginine	178 mg
Lysine	149 mg
Isoleucine	134 mg
Methionine	152 mg
Threonine	122 mg
Glycine	77 mg
CEM4	
Distilled water	125 mg
$ZnSO_4 \cdot 7H_2O$	0.55 mg
$CoSO_4 \cdot 7H_2O$	0 12 mg
Na_2EDTA	2.68 mg

(*Continued*)

TABLE 1. Compositions of enrichment media. All concentrations used in the tests were 2% unless otherwise indicated. — (*Concluded*)

CEM4 modifications			
Distilled water 125 ml in all modifications			
	$ZnSO_4\cdot 7H_2O$ (mg)	$CoSO_4\cdot 7H_2O$ (mg)	Na_2EDTA (mg)
CEM4A	0.275	0.06	1.34
CEM4B	0.55	0.06	2.46
CEM4C	0.275	0.12	1.56
CEM4D	5.50	1.20	26.75
CEM4E	0.055	0.012	0.268
CEM4F	0.006	0.001	0.027
CEM4G	0.55	0.12	5.36
Antibiotic medium			
Penicillin G		200 mg	
Streptomycin		100 mg	
Distilled water		10 ml	
Chelating agent medium			
Na_2EDTA		2.68 mg	
Distilled water		125 ml	

chemicals that were providing the benefit in CEM1 various groups were deleted. When the results of removing one of these groups indicated that its components had increased survival, further division of the group was made to test individual or small groups of chemicals. The major groups of CEM1 components tested were the vitamins and trace metals. The division of these groups into smaller units was by possible function in the case of vitamins and by valence in the case of trace metals. Thus vitamins associated with what could loosely be termed construction were lumped together (group 1), others were combined because of their association with chemical processes dealing with the release of energy or their association with coenzyme A (group 2), and the third group consisted of those vitamins frequently associated with the reduction of the size of organic molecules. The trace metals were divided into two groups, the first comprising the polyvalent ions (group 1), the second the normally divalent ions although boron, which is not normally considered a metal, was included in this group. Ultimately, the individual chemicals that were beneficial were combined and the effect compared with that of the original medium. Various concentrations of the beneficial chemicals were also tested to determine the optimum levels.

In addition to CEM1 and its modifications, two additional media were tested. The first (CEM3, Table 1) contained 12 amino acids; the second (antibiotic medium in Table 1) was used to test the effect of two antibiotics on the egg and naupliar stages. Because of insufficient testing, as well as the problem with antibiotics mentioned in the section Materials and Methods, the antibiotic medium (Tables 1 and 3) should be retested.

INTERPRETATION AND ANALYSIS OF RESULTS

The tests for any one month included the rearing of eggs and naupliar stages in sea water collected that month as well as in the same sea water enriched by various media. The test of the sea water alone provided a reference for comparison of the media used at that time. The effects of the unmodified sea water also indicated the tolerance of each stage to that type of sea water. The enrichment media were evaluated by comparison with the unmodified sea water and with other enrichment media used during the month.

In the comparisons of the various media (Tables 2–8) the percentage of survival in each stage was used. The egg and first nauplius stage were combined for analysis because approximately 90% of the eggs hatch directly into the second naupluis, the first being passed within the egg.

The method used for the comparisons was developed with the aid of Dr P. L. LeBlond of the Institute of Oceanography, University of British Columbia. It consists of dividing the percentage of survival, of each stage, in the test medium by the same value obtained in the medium with which it is being compared. Without modifications, this value equates beneficial and detrimental effects when plotted on two cycle semilogarithmic graph paper. For general comparisons and discussions, however, it was found best to use zero as the level at which the test exhibits neither benefit nor detriment. To do this, 1 is subtracted from the value obtained by the division. Thus, a plus value would indicate benefit and a minus value detriment. The main criticism of this technique is that it does not incidate the actual values of survival. To reduce this problem, the numbers of eggs and of sixth nauplii that moulted into the first stage copepodite are also given.

RESULTS AND DISCUSSION

The egg + N1 stage is the most critical part of early development of *E. japonica* (Fig. 2). This is followed by the N2, N6, N5, N3, and N4 stages with the last exhibiting the highest average survival. The egg to N2 stages are pre-feeding stages and appear to be dependent upon dissolved materials in the environment to supplement those not available or in low concentration in the deutoplasm or other stored nutrients. The N3 to N6 stages are feeding stages and the higher survival is felt to be due, at least in part, to the nutrient supply in the sea water. The N6, however, moults into the first copepodite, a change which is abrupt enough that Campbell (1934) termed it a metamorphosis. Whether or not the term is applicable is open to question but the preparation for the change may impose requirements on the stage that are not faced by the other feeding stages.

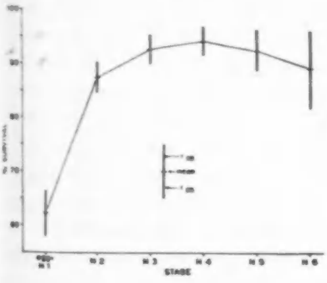

FIG. 2. Per cent survival of the early developmental stages in unenriched sea water. The 5% confidence limits are given with each mean.

The benefit of CEM1 is in increasing the survival of the early stages (Table 2). This benefit, however, varied on both a seasonal and a yearly basis, the periods of benefit being different in each of the 3 years tested although always occurring sometime during the winter or spring. That the stages most benefitted were the egg and N1 suggests, with the seasonal nature, that the effect could be due to a change in the nature of the water, the nature of the egg, or both. If it were the nature of the egg it could be in the amount of stored nutrients, which depends on the amount and nature of the food available to the egg-producing female. The period of maximum egg production occurs during the srping (Fig. 3), at a time when large numbers of developmental

TABLE 2. Effects of CEM1 on survival of *E. japonica*, in relation to survival in unenriched sea water.

Values given are: $\dfrac{\%\ \text{survival in CEM1}}{\%\ \text{survival in unenriched sea water}} - 1.$

Date	No. of eggs T/C[a]	No. of C1 T/C	Egg + N1	N2	N3	N4	N5	N6	Egg - N6
1966									
Jan.	47/98	31/36	+.30	+.02	+.07	+.18	+.07	0.00	+ .80
Apr.	289/140	97/12	+.97	+.41	0.00	−.07	+.08	+.38	+2.92
May	129/72	33/4	+.59	+.13	+.40	+.23	+.11	+.34	+3.60
June	410/138	144/38	+.33	+.02	−.07	−.02	+.07	+.31	+0.73
July	136/136	60/76	+.09	−.10	−.03	−.10	−.04	−.14	−0.21
Sept.	133/133	24/71	−.07	0.00	+.06	−.04	−.37	−.42	−0.66
Nov.	108/107	40/54	+.06	+.08	−.13	−.09	−.06	−.13	−0.27
Dec.	100/95	30/54	−.05	+.05	−.12	−.02	−.08	−.34	−0.47
1967									
Jan.	100/102	48/47	+.18	0.00	−.02	−.07	−.06	+.02	+ .04
Feb.	107/102	56/45	+.28	+.01	−.07	+.03	0.00	−.05	+0.19
Mar.	132/124	41/45	+.51	−.19	−.16	−.03	−.03	−.10	−0.14
Apr.	151/148	12/74	+.13	−.05	−.23	−.48	−.40	−.37	−0.84
May	33/75	0/31	−.12	−.07	−.13	−.17	−.18	−.94[b]	-
June	66/145	32/89	−.08	−.04	0.00	−.07	−.02	−.03	−0.21
July	64/137	14/71	+.05	−.03	−.22	−.04	−.11	−.38	−0.58
Aug.	74/139	4/65	−.40	−.03	−.38	−.31	0.00	−.55	−0.88
Sept.	121/120	16/45	+.19	+.07	−.25	−.22	−.26	−.35	−0.65
Oct.	60/115	13/60	0.00	−.17	−.02	−.31	+.06	−.31	−0.58
Nov.	55/102	14/41	+.12	−.17	−.06	−.08	0.00	−.22	−0.37
Dec.	41/89	8/22	+.13	−.07	+.02	−.23	−.07	+.03	−0.21
1968									
Jan.	41/49	11/24	−.12	−.19	+.01	0.00	−.13	−.11	−0.45
Feb.	55/55	17/29	−.06	+.06	−.06	−.17	−.12	−.15	−0.41
Mar.	64/65	11/7	−.19	+.01	−.07	+.05	+.29	+.56	+0.60
Apr.	98/110	38/50	+.32	−.13	−.03	−.03	−.14	−.10	−0.15
May	80/76	3/38	−.02	−.29	−.19	−.22	−.61	−.57	−0.92
Total			+3.14	−0.67	−1.68	−2.28	−2.00	−3.62	

[a] T/C: number in test/nμmber in control.

[b] Percentage survival was 0; value given is: $\dfrac{\%\ \text{mortality in unenriched sea water}}{\%\ \text{mortality in CEM1}} - 1.$

stages of the copepod *Calanus plumchrus* Marukawa are available as a food source (A.S. Pandyan, unpublished data). This increase in food to the female, however, does not provide any demonstrable increase in the overall survival of the eggs. This suggests that the seasonal abundance of food is primarily responsible for the number of eggs produced and not their viability.

The increase in the survival effected by CEM1 was presumably due to one or more substances in the medium. Comparison of the effects of deficient media in relation to those of the complete medium (CEM1A to CEM1I,

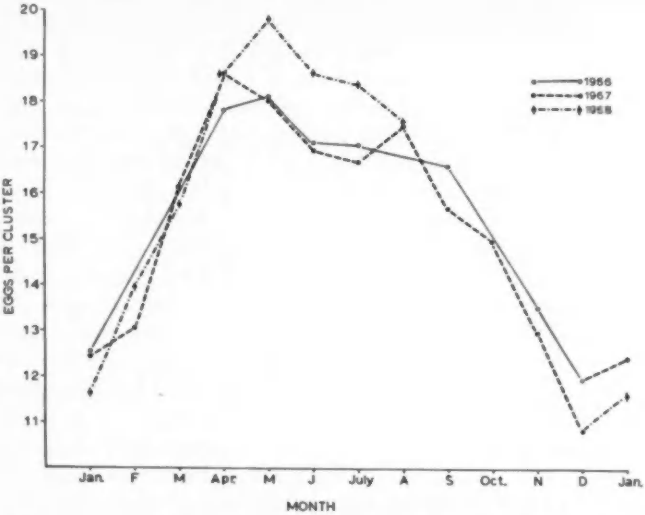

FIG. 3. Average numbers of eggs per egg cluster from January 1966 through August 1968. Each point represents the average of 32 egg clusters.

Table 4) indicated that the benefit was primarily due to zinc (CEM1G) and cobalt (CEM1H). The recombination of the two chelated trace metals, as a separate medium (CEM4, Table 1), gave higher survival than CEM1 (CEM4, Table 4). Comparison of the results of using CEM4 with those of unenriched sea water (Table 5) indicates a seasonal increase in the survival of the early developmental stages similar to but for a longer period than the increase effected by CEM1. Comparison of the survival given in Table 4 with that of the same months for CEM1 (Table 2, or CEM4 in Table 4) indicates that not only are the three components of CEM4 beneficial but also that one or more of the components of CEM1 are detrimental.

The studies of Shiraishi and Provasoli (1959), Provasoli and Shiraishi (1959), Flückiger and Flück (1950), and others (see Provasoli, 1963) have shown that certain dissolved organics are beneficial to the cultures of several crustaceans. This, in addition to the indication by Stephens and Schinske (1961) that various dissolved amino acids could be taken up by many marine invertebrates other than crustaceans, formed the basis for testing the effect of dissolved organics on the survival of the early developmental stages. With the exception of nicotinamide, the organics used had been shown to be beneficial to other crustaceans, nicotinamide being used to provide not only nicotinic acid, as one of the vitamins, but also its amide. With the exception of one test with amino acids (CEM3, Table 1), the organics were added to CEM1 in order to compare the results with those obtained by using unaltered CEM1. In all instances, the overall effect was to reduce the survival from that obtained with CEM1 (Table 3). In the case of the medium containing amino acid

TABLE 3. Effects of several organics on survival, in relation to survival in CEM1. Values given are: $\frac{\%\text{ survival in test medium}}{\%\text{ survival in CEM1}} - 1.$

Date (*1966*)	Medium tested	No. of eggs T/C[a]	No. of C1 T/C	Egg + N1	N2	N3	N4	N5	N6	Egg – N6
Apr.	CEM2	141/289	21/97	+.08	–.07	–.01	–.10	+.03	–.52	–0.56
May	CEM2	78/129	1/33	+.17	–.36	–.57	–.08	+.13	–.25	–0.95
Sept.	CEM1 + vit. E	133/133	13/24	–.26	–.09	–.31	–.26	+.19	+.29	–0.46
Nov.	CEM3	104/108	0/40	–.32	–.40	–.53	–.66	–.94[b]	–	–
Nov.	CEM1 + nicotinamide	55/108	10/40	–.14	–.04	–.16	–.09	–.22	–.29	–0.51
Dec.	CEM1 + CEM3[c]	96/100	6/30	+.14	–.07	–.13	–.58	–.36	–.15	–0.79
Dec.	Antibiotics[c]	92/100	12/30	+.03	–.01	+.03	–.34	–.46	–.08	–0.57

[a] T/C: number in test/number in control.

[b] Survival was 0; value given is: $\frac{\%\text{ mortality in CEM1}}{\%\text{ mortality in test}} - 1.$

[c] CEM3 concentration = 0.2%; antibiotic concentration = 1%.

(CEM3), G. C. Stephens (personal communication) felt that the concentration was too high. As a result of this, a concentration an order of magnitude less was combined with CEM1 (Table 3) which provided some benefit to the egg + N1 stage but not to the early development as a whole.

The lack of benefit by the organics added to the CEM1 indicates that if the tested organics were beneficial there was some factor affecting the expression of their benefit. This is further indicated by the slight increase in overall benefit when some of the vitamins were removed from CEM1 (CEM1D, Table 4) as well as the small overall detrimental effect when the additional vitamins were deleted (CEM1B, 1C in Table 4). The reaction to organics may be the result of bacteria and the increase in bacterial growth presumably caused by the organic fraction of the enrichment media. This is based on the difference in the number of bacteria in samples taken at the beginning and end of a 3-day period (initial concentration approximately 100 per ml, final concentration 4500 per ml in unenriched sea water, 25,000 per ml, in CEM1-enriched sea water) as obtained with the nutrient agar medium described by ZoBell and Anderson (1936).

The determination of the beneficial chemicals in CEM1 by testing deficient media as well as the benefit provided by CEM4 indicated the effect of chelated cobalt and zinc in increasing the survival. It did not, however, provide an indication of the effect of the chelated trace metals used individually. The results of testing chelated zinc and cobalt individually in the amounts used in CEM1 (Table 6; Zn + Chel. Ag.; Co + Chel. Ag.) indicate that each trace metal does not provide the benefit of both together.

The seasonal variations in the benefits of CEM1 and CEM4 suggested that the naturally occurring amounts of zinc and cobalt might vary with time. Besides suggesting the possibility of these trace metals being a limiting factor in development, the variations indicated the need for determining the optimum amounts, or optimum ratios of the ions, for the various stages. To obtain this, it was necessary to determine the amounts present in the sea water. The amount given for February 1967 (Table 6) was obtained with a Techtron AA-100 atomic absorption spectrophotometer, through the sea water concentration technique described by Joyner et al. (1967). Complete recovery of cobalt, as judged through the use of standards, was never achieved and the results were not reproducible. (A second trace metal concentration technique, suggested by E.V. Grill (personal communication), has been developed but has not yet been applied to a sufficient number of seawater samples to allow adequate evaluation.) Because no specific determinations were made for the sea water, the results given in Table 6 (CEM4A–CEM4G) can only be used with reservation. They do suggest, however, that the benefit from unmodified CEM4 is not substantially changed by varying the amounts of the trace metal components. In two of the three overall values higher than that for CEM4 (CEM4D, CEM4G for April) the media had higher concentrations of chelating agents, relative to the amounts of trace metals, suggesting some importance for this part of the medium.

TABLE 4. Effects of CEM1 modifications on survival, in relation to survival in CEM1. Values given are: $\frac{\text{\% survival in test medium}}{\text{\% survival in CEM1}} - 1.$

Date	Medium tested	No. of eggs T/C[a]	No. of C1 T/C	Egg + N1	N2	N3	N4	N5	N6	Egg − N6
1967										
Feb.	CEM1A	104/107	6/56	−.05	−.21	−.18	−.37	−.07	−.69	−0.89
Feb.	CEM1B	105/107	54/56	+.01	+.03	−.04	0.00	−.01	0.00	−0.02
Mar.	CEM1C	65/132	9/41	−.06	+.05	+.04	+.02	−.06	−.54	−0.55
"	CEM1D	64/132	21/41	+.16	+.22	+.28	0.00	−.20	−.16	+0.06
"	CEM1E	65/132	16/41	+.01	+.10	+.13	−.06	−.18	−.19	−0.21
"	CEM1F	66/132	1/41	−.13	+.15	+.04	−.29	−.68	−.78	−0.95
Apr.	CEM1G	76/151	2/12	−.22	−.14	+.22	−.26	−.25	−.27	−0.67
"	CEM1H	73/151	0/12	−.04	−.23	−.21	+.10	−.70	−.55	−0.08[b]
"	CEM1I	74/151	3/12	0.00	−.25	+.01	+.66	−.48	−.22	−0.49
May	CEM4	35/33	7/0	−.52	+.23	+.32	+.30	+.17	+3.55[b]	+4.00[b]
June	CEM4	68/66	39/32	−.06	+.10	+.01	+.09	0.00	+.03	+0.18
July	CEM4	60/64	20/14	−.24	−.04	+.25	0.00	+.14	+.49	+0.52
Dec.	CEM4	89/41	29/8	−.21	+.16	+.02	+.35	+.08	+.21	+0.67
1968										
Jan.	CEM4	46/41	31/11	+.25	+.41	+.06	−.03	+.20	+.14	+1.51
Feb.	CEM4	70/55	49/17	+.33	+.02	+.04	+.13	+.20	+.15	+1.26
Mar.	CEM4	75/64	18/11	+.26	+.02	+.19	+.14	+.03	−.20	+0.40
Apr.	CEM4	116/98	63/38	+.05	+.09	−.03	−.04	+.13	+.17	+0.40
May	CEM4	81/80	38/3	+.19	+.38	+.14	+.22	+1.39	+1.33	+11.51

[a] T/C: number in test/number in control.

[b] Survival in test medium or CEM1 was 0; value given is: $\frac{\text{\% mortality in CEM1}}{\text{\% mortality in test}} - 1.$

TABLE 5. Effects of CEM4 on survival, in relation to survival in unenriched sea water. Values given are: $\frac{\%\text{ survival in CEM4}}{\%\text{ survival in unenriched sea water}} - 1.$

Date	No. of eggs T/C*	No. of C1 T/C	Egg + N1	N2	N3	N4	N5	N6	Egg − N6
1967									
May	35/75	7/31	−.49	+.15	+.15	+.08	−.04	−.17	−0.52
June	68/145	39/89	−.14	+.06	+.01	+.01	−.02	0.00	−0.07
July	60/137	20/71	−.21	−.07	−.03	−.04	+.01	−.08	−0.36
Dec.	89/89	29/22	−.10	+.08	+.04	+.04	+.01	+.25	+0.32
1968									
Jan.	46/49	31/24	+.10	+.14	+.08	−.03	+.04	+.01	+0.38
Feb.	70/55	49/29	+.25	+.08	−.02	−.06	+.06	−.02	+0.33
Mar.	75/65	18/7	+.01	+.03	+.10	+.19	+.32	+.25	+1.23
Apr.	116/110	63/50	+.38	−.05	−.06	−.07	−.03	+.05	+0.19
May	81/76	38/38	+.17	−.02	−.07	−.05	−.07	0.00	−0.06
June	95/110	44/44	+.18	+.03	−.07	−.06	+.06	0.00	+0.16
July	109/105	46/53	−.03	−.04	−.11	+.01	−.01	0.00	−0.16

*T/C: number in test/number in control.

The function of chelating agents is to "... effectively bring the very sparingly soluble metals into solution" (Johnston, 1964, p. 88). Taylor (1964) indicates that one chelating agent at least, may also form a source of organic carbon for various members of the phytoplankton. The intended role of the chelating agent in the CEM series was to act upon the added trace metals but they also presumably acted upon the naturally occurring trace metals (see Provasoli, 1963, for an excellent review of the effect of chelating agents). When the chelating agent was used by itself as an enrichment agent (Table 1), development was benefitted except during 3 months (Table 7). The role that the chelating agent played in CEM4 was determined by comparing the effects of the chelating agent with those of the CEM4 run at the same time. These results (Table 8) indicate that direct benefit was provided by the addition of chelated trace metals only during 2 months (January, February 1968). There is also some evidence (Table 8: July 1968) that the chelating agent may be detrimental when there is an abundance of chelatable agents as has been suggested (Provasoli, 1963, p. 201) for phytoplankton.

Although the pre-feeding stages derived the greatest benefit from the trace metals in CEM4 (Table 8), some of the values for CEM1 show a benefit for the feeding stages (Table 2 : January, April, May, and June 1966; March 1968). These were for periods when the enrichment medium was most effective and suggest that there were low amounts of naturally occurring materials. This also indicates that all of the nutrients are not supplied by the food and that the feeding stages rely, in part, on dissolved materials. This is

TABLE 6. Effects of CEM4 modifications on survival, in relation to survival in CEM4. Values given are: $\frac{\%\text{ survival in test medium}}{\%\text{ survival in CEM4}} - 1.$

Date (*1968*)	Medium tested	No. of eggs T/C[a]	No. of C1 T/C	Egg + N1	N2	N3	N4	N5	N6	Egg – N6
Jan.	Zn + Chel. Ag.	75/46	39/31	+.01	–.23	–.02	+.03	–.02	0.00	–0.23
Jan.	Co + Chel. Ag.	73/46	41/31	–.09	–.06	–.07	+.03	+.03	–.02	–0.17
Feb.[b]	CEM4A	71/70	48/49	+.04	–.04	–.05	+.04	0.00	0.00	–0.03
"[b]	CEM4B	68/70	40/49	–.11	–.06	0.00	+.04	0.00	0.00	–0.16
"[b]	CEM4C	69/70	50/49	–.04	–.02	+.02	+.06	0.00	+.02	+0.04
Mar.	CEM4D	79/75	24/18	+.04	–.10	–.07	–.11	+.01	+.63	+0.27
"	CEM4E	79/75	15/18	–.11	–.11	+.01	–.02	–.04	+.05	–0.21
"	CEM4F	79/75	12/18	–.17	–.12	–.06	–.07	–.12	+.10	–0.37
Apr.	CEM4G	98/116	64/63	–.04	+.07	+.05	+.08	+.01	+.02	+0.20
May	CEM4G	74/81	31/38	–.16	–.15	+.08	–.04	+.08	0.00	–0.11

[a] T/C: number in test/number in control.

[b] Analysis of February sea water from IOUBC/GS-1 indicates an approximate concentration of: cobalt at 1.18 μg per liter; zinc at 13.86 μg per liter. See text for discussion of analysis.

TABLE 7. Effects of a chelating agent (Na_2EDTA) on survival, in relation to survival in unenriched sea water.

Values given are: $\dfrac{\%\ \text{survival in chelating agent}}{\%\ \text{survival in unenriched sea water}} - 1.$

Date	No. of eggs T/C[a]	No. of C1 T/C	Egg + N1	N2	N3	N4	N5	N6	Egg − N6
1967									
Sept.	125/120	52/45	+.22	0.00	−.02	+.04	−.03	−.08	+0.11
Oct.	126/115	38/60	0.00	−.09	+.04	−.31	−.06	−.55	−0.42
Nov.	54/102	24/41	+.38	−.05	−.04	+.04	−.10	−.06	+0.11
Dec.	40/89	16/22	+.30	+.15	+.04	+.02	−.07	+.12	+0.62
1968									
Jan.	42/49	22/24	+.03	+.02	+.07	−.12	+.08	0.00	+0.07
Feb.	57/55	27/29	−.06	−.08	−.04	0.00	+.06	0.00	−0.10
Mar.	64/65	17/7	−.29	−.07	+.13	+.12	+.53	+.97	+1.47
Apr.	83/110	56/50	+.27	0.00	0.00	+.04	0.00	+.05	+0.48
May	76/76	40/38	+.22	−.12	+.01	−.02	0.00	0.00	+0.05
June	97/110	51/44	+.39	−.01	−.08	−.01	−.01	+.06	+0.31
July	115/105	48/53	+.16	−.10	−.01	−.08	−.06	−.06	−0.17

[a] T/C: number in test/number in control.

TABLE 8. Effects of the trace metals in CEM4 on survival, in relation to survival in chelating agent (Na_2EDTA).

Values given are: $\dfrac{\%\ \text{survival in CEM4}}{\%\ \text{survival in chelating agent}} - 1.$

Date	No. of eggs T/C[a]	No. of C1 T/C	Egg + N1	N2	N3	N4	N5	N6	Egg − N6
1967									
Dec.	89/40	29/16	−.31	−.06	0.00	+.06	+.08	+.10	−0.19
1968									
Jan.	46/42	31/22	+.07	+.11	0.00	+.10	−.03	+.01	+0.29
Feb.	70/57	49/27	+.33	+.17	+.02	−.06	0.00	−.02	+0.48
Mar.	75/64	18/17	+.42	+.11	−.02	+.06	−.13	−.37	−0.10
Apr.	116/83	63/56	+.09	−.05	−.06	−.11	−.03	0.00	−0.20
May	81/76	38/40	−.04	+.12	−.08	−.03	−.07	0.00	−0.11
June	95/97	44/51	−.15	+.05	+.01	−.03	+.08	−.06	−0.12
July	109/115	46/48	−.16	+.07	−.10	+.10	+.06	+.06	+0.01[b]

[a] T/C: number in test/number in control.

[b] Value an artifact in indicating benefit to survival because development to N6 was not benefitted by CEM4 during July (Table 5).

substantiated by the March 1968 values in Table 7 which show the increase in survival of the feeding stages effected by the presence of a chelating agent. Comparing these with the March 1968 values in Table 8 suggests the possibility that agents other than zinc and cobalt are beneficial to the feeding stages. The overall detriment to the survival evidenced by the organics tested, and the beneficial effect of the chelating agent, suggest that chelatable inorganics in addition to those used in CEM4 and probably other than those others found in CEM1 are of benefit to the early developmental stages of *E. japonica.*

REFERENCES

AUTIAN, J. 1964. Toxicity, untoward reactions, and related considerations in the medical use of plastics. J. Pharmacol. Sci. 53: 1289–1301.

BARY, B. MCK. 1963. The distribution of Atlantic pelagic organisms in relation to surface water bodies, p. 51–67. *In* M. J. Dunbar [ed.] Marine distributions. Roy. Soc. Canada, Spec. Publ. 5.

BERNHARD, M., A. ZATTERA, AND P. FILESI. 1966. Suitability of various substances for use in the culture of marine organisms. Pubbl. Staz. Napoli 35: 89–104.

CAMPBELL, M. H. 1934. The life history and post embryonic development of the copepods, *Calanus tonsus* Brady and *Euchaeta japonica* Marukawa. J. Biol. Bd. Canada 1: 1–65.

DAVIS, C. C. 1949. The pelagic Copepoda of the northeastern Pacific Ocean. Univ. Wash. Publ. Biol. 14: 1–118.

FLÜCKIGER, E. AND H. FLÜCK. 1950. Der Einfluss von Vitamin E auf *Daphnia longispina* O.F.M. Experientia 6: 64.

FULTON, J. MS, 1968. A laboratory manual for the identification of British Columbia marine zooplankton. Fish. Res. Bd. Canada, Tech. Rept. 55. 141 p.

JOHNSTON, R. 1964. Sea water, the natural medium of phytoplankton. II. Trace metals and chelation, and general discussion. J. Marine Biol. Assoc. U.K. 44: 87–109.

JOYNER, T., M. L. HEALY, D. CHAKRAVARTI, AND T. KOYANAGI. 1967. Preconcentration for trace analysis of sea waters. Environ. Sci. Technol. 1: 417–424.

LEWIN, R. A. [ed.]. 1962. Physiology and biochemistry of algae. Academic Press, Inc., New York. 929 p.

LEWIS, A. G. 1967. An enrichment solution for culturing the early developmental stages of the planktonic marine copepod *Euchaeta japonica* Marukawa. Limnol. Oceanog. 12: 147–148.

MARSHALL, S. M., AND A. P. ORR. 1958. Some uses of antibiotics in physiological experiments in sea water. J. Marine Res. 17: 341–346.

PROVASOLI, L. 1963. Organic regulation of phytoplankton fertility. *In* M. N. Hill [ed.] The sea. Vol. 2. Interscience; John Wiley and Sons, Inc., New York. 554 p.

PROVASOLI, L., AND K. SHIRAISHI. 1959. Axenic cultivation of the brine shrimp *Artemia salina*. Biol. Bull. 117: 347–355.

SHIRAISHI, K., AND L. PROVASOLI. 1959. Growth factors as supplements to inadequate algal foods for *Tigriopus japonicus.* Tohoku J. Agr. Res. 10: 89–96.

STEPHENS, G. C., AND R. A. SCHINSKE. 1961. Uptake of amino acids by marine invertebrates. Limnol. Oceanog. 6: 175–181.

TAYLOR, W. R. 1964. The uptake of ethylenediamine tetraacetic acid by pure cultures of marine plankton algae. Biol. Bull. 127: 392–393.

WALDICHUK, M. 1957. Physical oceanography of the Strait of Georgia, British Columbia. J. Fish. Res. Bd. Canada 14: 321–486.

WILSON, D. P. 1951. A biological difference between natural sea waters. J. Marine Biol. Assoc. U.K. 30: 1–26.

WILSON, D. P., AND F. A. J. ARMSTRONG. 1952. Further experiments on biological differences between natural sea-waters. J. Marine Biol. Assoc. U.K. 31: 335–349.

ZOBELL, C. E., AND D. Q. ANDERSON. 1936. Observations on the multiplication of bacteria in different volumes of stored sea water and the influence of oxygen tension and solid surfaces. Biol. Bull. 71: 324–342.

NOTES

Productivity of Owikeno Lake, British Columbia

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Although Owikeno Lake is the third highest producer of adult sockeye salmon in North America in terms of mean annual total return (catch plus escapement) per unit of lake nursery area, limited measurements of other indices of lake productivity suggest that its primary productivity is much lower than that of the other four highest sockeye-producing lakes. The implications of these results to sockeye production are discussed.

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Owikeno Lake is located on the central coast of British Columbia and among major sockeye salmon (*Oncorhynchus nerka* (Walbaum)) lakes in North America is the third highest producer in terms of the mean annual total return (catch plus escapement) per unit of lake nursery area. The productivity of this lake supports a major commercial fishery; the mean annual catch was about 1,015,000 from 1912 to 1947 and 824,000 from 1948 to 1967. The highest recorded catch occurred in 1968 and totalled 2.7 million. Estimates of escapements, based on limited spawning ground counts, averaged 476,000 from 1948 to 1967 (Canada Department of Fisheries, Pacific Region).

Owikeno Lake is essentially a 56-km extension of a coastal fiord comprised of several deep basins and with limited littoral area (Ruggles, 1965; Foskett, 1958). It lies in a region of heavy precipitation, over 380 cm average annually, and water levels fluctuate as much as 7 m during a given year. The lake and most of its major tributaries are turbid with glacial silt throughout the summer.

Despite the relatively high production of adults, young sockeye grow slowly in the lake, and growth during the 1st year is apparently density-dependent (Ruggles, 1965). Virtually all sockeye smolts from Owikeno Lake are yearlings and are small compared with those migrating from other lakes in British Columbia (Ricker, 1966). Scales of these smolts usually show appreciable growth after the annulus, thus apparently much of their growth is obtained in the lake just before migration from the lake (Ruggles, 1965).

In 1967, an opportunity was presented to obtain limited measurements of primary productivity in Owikeno Lake. The purpose of this note is to present these measurements, to compare them with similar but more extensive

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measurements made for other major sockeye-producing lake systems, and to discuss their implications in sockeye production.

Primary production methods — Measurements of primary production were made at two stations about 10 km apart on Owikeno Lake on June 24 and September 18, 1967.

Photosynthetic activity was measured by carbon 14 uptake using methods outlined by Narver (1967) except where noted here. $Na^{14}CO_3$ had a specific activity of 5 μc/ml. Filtration was accomplished with a hand-operated vacuum pump. Incubation was in situ for 4 hr from approximately 1000 to 1400 hr. Total alkalinity and *p*H were measured with portable Hach water analysis equipment (Model Al-36-WR). Chlorophyll *a* was determined after the method of Strickland and Parsons (1965). The two stations were located in basins I and II because the greatest densities of young sockeye occur in these basins (Ruggles, 1965).

The primary production data for Alaskan lakes were collected in 1962 (Burgner et al., 1968) and for Babine Lake in 1966 (Narver, 1967) and 1967. It is assumed (1) that measurements made at two stations on 2 days, 2 or 3 months apart indicate the general magnitude of primary production; and (2) that annual variation does not introduce serious error in this comparison of magnitudes of primary production. In light of experience in some large Alaskan sockeye lakes the assumptions appear justified (R.L. Burgner, personal communication).

Results — Measurements of primary production made in Owikeno Lake in 1967 are presented in Table 1. Three features are immediately apparent: (1) carbon assimilation was much higher at station 1 on June 24 than at the other station or sampling date; the associated standing crop of phytoplankton as indicated by chlorophyll *a* was also somewhat larger on that date than at station 2; (2) chlorophyll *a*, total alkalinity (and therefore available carbon), and total dissolved solids were higher on September 18 than June 24; and (3) the high turbidity of Owikeno Lake was reflected in the shallow Secchi and compensation depths.

Owikeno Lake ranked third behind Chignik and Karluk lakes, Alaska, in terms of mean annual total return of adult sockeye salmon per km² of nursery lake area (Table 2). However, Owikeno Lake (in 1967) was generally much lower in rate of carbon assimilation (and the related *p*H and total alkalinity), total dissolved solids, and chlorophyll *a* than the Alaskan lakes (in 1962).[1]

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[1] Dr Loyd A. Royal, Director of the International Pacific Salmon Fisheries Commission, kindly provided minimum total run (catch plus escapement) data from 1952 to 1967 for the most productive sockeye salmon lakes in the Fraser River system, British Columbia. On an *annual* basis Cultus (a small lake), Fraser, and Shuswap lakes all rank with the top five lakes in Table 2.

Lake	Annual mean total return/km²	Area km²
Cultus	18,000	6
Fraser	8,000	52
Shuswap	7,400	310
Bowron	5,300	11
Chilko	5,100	94

TABLE 1. Primary productivity and associated measurements of Owikeno Lake in 1967.

	Chlorophyll *a* (*mg/m*³)	C assimilated/4 hr		*p*H	Total alkalinity (*ppm* $CaCO_3$)	Available C (*mg/liter*)	Secchi (*m*)	Comparative depth (*m*)	Total dissolved solids (*ppm*)	Surface temp (*C*)
		(*mg/m*³)	(*mg/m*²)							
June 24										
Station 1	.68	30.23	3.33	6.6	10.3	4.20	1.0	9	22.5	11.0
Station 2	.27	4.54	1.00	6.7	10.3	4.20	.7	4	18.6	12.5
Sept. 18										
Station 1	1.18	2.57	.62	6.7	15.9	6.05	.5	3	23.3	12.8
Station 2	1.34	4.85	1.46	6.7	17.1	6.49	.5	3	22.0	11.8

TABLE 2. Mean indices of primary production of the five most productive sockeye salmon nursery lakes in North America in terms of mean annual total return of adults per km^2 of lake surface, and comparative data for Babine Lake, B.C.

Lake	Annual mean total return/km^2	Area (km^2)	Year	Expt. no.	Chlorophyll *a* (mg/m^3)	C assimilated /4 hr		pH	Total alkalinity (ppm $CaCO_3$)	Available C ($mg/liter$)	Secchi (m)	Total dissolved solids (ppm)
						(mg/m^2)	(mg/m^3)					
Chignik, Alaska[a],[b]	26,200 (1949–64)	24	1962	8	15.3	242	13.5	7.6	21.3	6.0	1.9	55.9
Karluk, Alaska[a],[c]	16,000 (1950–67)	40	1962	–	2.1	131	5.9	7.2	25.2	–	8.6	42.9
Owikeno, B.C.	13,500 (1948–67)	96	1967	4	.9	11	1.8	6.7	13.4	5.2	.7	21.6
Igushik, Alaska[a],[d] (2 lakes)	7,000 (1956–66)	67	1962	–	.8	73	2.5	7.2	10.7	–	9.1	19.5
Black, Alaska[a],[b]	6,800 (1949–64)	39	1962	8	5.4	121	33.4	7.4	21.5	5.4	1.6	69.7
Babine, B.C.[e,f]												
North Arm–Nilkitkwa	8,100 (1957–67)	56	1966	4	–	21	2.3	7.4	32.9	8.8	3.5	–
			1967	3	.6	14	1.7	7.3	34.3	9.3	3.3	69.5
Main Lake	1,700 (1957–67)	430	1966	10	–	22	1.8	7.2	33.0	9.4	4.1	–
			1967	4	.5	21	2.4	7.4	34.4	9.6	4.4	67.9

[a] Primary production data for 1962 (Burgner et al., 1968).

[b] Total run data from Narver (MS, 1966).

[c] Total run data from Mr B. Drucker (personal communication, U.S. Bureau of Commercial Fisheries, Auke Bay, Alaska).

[d] Total run data from Ossiander (1967).

[e] Annual catch of Babine Lake sockeye is taken as 93% of the Skeena River catch (the 10-year mean Babine Lake portion of the total Skeena River sockeye escapement). Mean number of sockeye produced in North Arm–Nilkitkwa is 36.5% of the Babine total (the 10-year mean North Arm–Nilkitkwa portion of the total Babine Lake escapement). Escapement data is from Department of Fisheries of Canada (MS, 1965) and Mr H. W. Smith (personal communication, Nanaimo, B.C.)

[f] Primary production data for 1966 (Narver, 1967).

Babine Lake is the only major sockeye salmon lake in British Columbia for which primary production data exist. In terms of density of young sockeye, Babine Lake has two quite different regions: the North Arm–Nilkitkwa region has an average (1957–63) October density that is almost five times that of the main lake region (Department of Fisheries of Canada, MS, 1965). The North Arm–Nilkitkwa region has an annual mean rate of adult sockeye production that is about three-fourths that of Owikeno Lake; sockeye production in the main lake region of Babine Lake is much lower (Table 2). Mean indices of primary production from 1966 and 1967 in both regions of Babine Lake can be compared with similar measurements made in Owikeno. A difference in rate of carbon assimilation between the two lakes is difficult to demonstrate (Table 1, 2). Babine Lake is markedly higher in total dissolved solids; Owikeno Lake had a higher chlorophyll *a* content and much lower Secchi depth (Table 2). (Differences between 1966 and 1967 within either region of Babine Lake were rather small.)

Discussion — The limited measurements made in Owikeno Lake in 1967 suggest a low rate of primary production compared with some other major sockeye-producing lakes for which there are comparable data. The high turbidity and low total dissolved solids of Owikeno Lake are probably important contributing factors. If these estimates made in Owikeno Lake are representative of the general level of primary production, the slow growth of young sockeye could be explained by an equally slow rate of energy transfer through the phytoplankton–zooplankton–sockeye food chain. The relatively large return of adults compared with other sockeye watersheds may reflect exceptionally low mortality rates and perhaps a situation contrary to the smolt size–marine mortality relation postulated by Ricker (1962). Predators, at least those that feed by sight, are probably not very efficient in the turbid waters of Owikeno Lake, Wannock River (the outlet), and Rivers Inlet. Furthermore, the close proximity of Owikeno Lake to the ocean (Wannock River is only 7 km long) eliminates the long, and presumably hazardous, downstream trip that the smolts of most stocks of sockeye salmon in British Columbia must make. Finally, the Owikeno Lake stocks have a scale pattern that suggests 1st year in the ocean growth is greater than that in all other sockeye stocks (Gilbert, 1914). This comparison suggests that these sockeye may have an extremely favourable nursery area for their early marine life.

Unfortunately, little is known about either the population dynamics of juvenile sockeye in Owikeno Lake or the magnitude and efficiency of trophic energy transfer underlying juvenile sockeye production. Furthermore, virtually nothing is known about the marine life history of the Owikeno Lake stocks of sockeye salmon. Without this understanding, the significance of primary production measurements relative to the rearing capacity of the lake for juvenile sockeye or to the magnitude and stability of adult sockeye numbers is unknown. Because of the economic importance as well as the biological uniqueness of these stocks of sockeye salmon, the entire question of primary, secondary, and tertiary production in the Owikeno Lake ecosystem, as well as the early marine life history, merits further investigation.

REFERENCES

BURGNER, R. L., C. J. DICOSTANZO, R. J. ELLIS, G. Y. HARRY, W. L. HARTMAN, O. J. KERNS, O. A. MATHISEN, AND W. F. ROYCE. 1968. Biological studies and estimates of optimum escapements of sockeye salmon in the major systems in southwestern Alaska. U.S. Bur. Comm. Fish. Bull. (In press.)

DEPARTMENT OF FISHERIES OF CANADA. MS, 1965. Proposed sockeye salmon development program for Babine Lake. Pacific Area, Vancouver, B.C. 53 p.

FOSKETT, D. R. 1958. The Rivers Inlet sockeye salmon. J. Fish. Res. Bd. Canada 15: 867–889.

GILBERT, C. H. 1914. Contribution to the life-history of sockeye salmon (No. 1). Rept. Comm. Fish. (1913): R53–R78.

NARVER, D. W. MS, 1966. Pelagial ecology and carrying capacity of sockeye salmon in the Chignik Lakes, Alaska. Ph.D. Thesis. University of Washington, Seattle, Wash. 348 p.
1967. Primary productivity in the Babine Lake system, British Columbia. J. Fish. Res. Bd. Canada 24: 2045–2052.

OSSIANDER, F. J. 1967. Bristol Bay red salmon forecast of run for 1967. Dept. Fish Game, Alaska. Information Leaflet 105. 51 p.

RICKER, W. E. 1962. Comparison of ocean growth and mortality of sockeye salmon during their last two years. J. Fish. Res. Bd. Canada 19: 531–560.
1966. Sockeye salmon in British Columbia. Intern. Comm. North Pacific Fish. Bull. 18: 59–70.

RUGGLES, C. P. 1965. Juvenile sockeye studies in Owikeno Lake, British Columbia. Canadian Fish Culturist 36: 3–21.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1965. A manual of sea water analysis. Bull. Fish. Res. Bd. Canada 125. 203 p.

Some Properties of the Fluorescence of Fish Muscle

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White muscle of a fish in prerigor condition fluoresces when irradiated with ultraviolet light of 366 mμ. Investigations carried out with the white sucker (*Catostomus commersoni*) indicate that the fluorescence is destroyed by treating the muscle with dilute acids. Emission spectra of fluorescence show a peak at about 460 mμ. Prerigor muscle also reduces a tetrazolium salt (Nitro BT) to its colored formazan. From these observations it is suggested that reduced pyridine nucleotides are at least partly responsible for the fluorescence of prerigor muscle.

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WHITE MUSCLE of a freshly killed fish shows visible fluorescence when irradiated with ultraviolet light of 366 mμ. The possibility of using this phenomenon as a test of the freshness of fish was recently indicated by Wittfogel (1967). He showed that in many fish species prerigor white muscle, either in fresh or frozen state, fluoresces under long wavelength ultraviolet light although it shows no fluorescence postrigor. Wittfogel stated that the cause of this fluorescence is "the property of lactic acid to emit a blue-white fluorescence on irradiation by black-filtered ultra-violet light." In the present note, results of a study of some of the properties of such fluorescence are given and it is suggested that reduced diphosphopyridine nucleotide (DPNH)[1] is at least partly responsible for it.

The fish used in this investigation were white suckers (*Catostomus commersoni*), which are very common in the lakes of central Canada. These were captured by gillnets and maintained in an aquarium for several days. When needed, they were removed from the aquarium by hand net and killed immediately by a blow on the head. The fish were then beheaded, gutted, and cleaned with cold tap water. A part of the white muscle was removed before the fish went into rigor and frozen at −20 C. Another portion of the white muscle was removed immediately after rigor was completely resolved, and frozen. In the case of an iced fish, it takes about 48 hr to establish rigor fully and about 48 hr more before rigor is completely resolved. If the fish is maintained at room temperature (25 C), rigor is established in about 12 hr and resolved in another 18 hr. When irradiated with ultraviolet light of 366 mμ, the prerigor muscle was readily distinguished by its fluorescence. Postrigor muscle showed no fluorescence.

The effect of dilute acids and alkalies on the fluorescence of prerigor muscle was studied by placing pieces of muscle in prepared solutions for 15 min at 4C; the muscle was then examined for fluoresecnce. Distilled water, 0.05 M phosphate buffer (pH 8.7), 0.1 N NaOH, and 0.1 M Na_2CO_3 had no adverse effect on the fluorescence; 0.1 N HCl, 0.1 N H_2SO_4, and 5% trichloroacetic acid destroyed the fluorescence completely.

The fluorescence emission spectrum of the muscle was determined with the use of a Beckman DK-2 recording spectrophotometer equipped with a fluorometer accessory. The peak of fluorescence for either frozen or unfrozen muscle was found to be at about 460 mμ (uncorrected). The intensity of fluorescence of the muscle after freezing, however, was always greater than that before freezing.

The destruction of fluorescence by acids and the peak of fluorescence at 460 mμ suggested a close similarity between the fluorescence of the muscle and that of DPNH. This compound is also destroyed in acids (Rafter and Colowick, 1957), and its fluorescence intensity is maximum at about 460 mμ (Kaplan 1960). Because of these similarities, a test used for the detection of DPNH was performed on both prerigor and postrigor muscle. The test, as described by Dewey and Conklin (1960), is useful in locating the activities of dehydrogenases in gel-electrophoresis procedures, and essentially detects DPNH. It involves the reduction of a soluble tetrazolium salt (Nitro BT) by DPNH to an insoluble purple formazan dye in the presence of phenazine methosulphate (PMS), which acts as an intermediate in the transfer of electrons from DPNH to Nitro BT. In the present study, pieces of prerigor and postrigor muscle were placed in an aqueous solution of Nitro BT and PMS (Nitro BT 50 mg/100 ml, PMS 5 mg/100 ml) at 25C for 60 min, and whether or not the muscle turned purple was noted. Prerigor muscle always became purple indicating that it reduced Nitro BT. Postrigor muscle, on the other hand, remained unchanged.

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[1] As the various chemical and physical properties of reduced diphosphopyridine nucleotide (DPNH), discussed in this note, are identical to those of reduced triphosphopyridine nucleotide (TPNH), whenever a reference is made to DPNH for its properties, it also applies to TPNH.

Jones and Murray (1966) observed that in "chill-stored" muscle of cod (*Gadus callarias*) the DPNH content decreased after death gradually over a period of 4 days. If the fluorescence shown by the fresh fish muscle is due to DPNH, then the decreasing concentration of DPNH will result in a gradual decrease in intensity of fluorescence. This is, indeed, what happens. It was observed in white suckers that the intensity of fluorescence decreases steadily from the time of death until rigor is resolved, after which there is no fluorescence.

The suggestion of Wittfogel (1967) that lactic acid is responsible for the fluorescence of fresh muscle is obviously incorrect. All evidence so far obtained by many workers shows that the lactic acid content in fish muscle increases continuously immediately after death (Tomlinson et al., 1961, Fraser et al., 1967); on the other hand, the muscle is always fluorescent at the time of death and nonfluorescent after the resolution of rigor when the lactic acid concentration is higher than that at the time of death. We did not notice any fluorescence when lactic acid solutions were irradiated with ultraviolet light of 366 mμ; however, DPNH is intensely fluorescent.

It is, therefore, suggested that DPNH is at least partly responsible for fluorescence of the fresh muscle. It is also evident that the property of prerigor muscle to give a positive reaction with Nitro BT is of value in differentiating prerigor muscle from a postrigor muscle in the early postmortem period.

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REFERENCES

DEWEY, M. M., AND J. L. CONKLIN. 1960. Starch gel electrophoresis of lactic dehydrogenase from rat kidney. Proc. Soc. Exptl. Biol. Med. 105: 492–494.

FRASER, D. I., J. R. DINGLE, J. A. HINES, S. C. NOWLAN, AND W. J. DYER. 1967. Nucleotide degradation, monitored by thin-layer chromatography, and associated postmortem changes in relaxed cod muscle. J. Fish. Res. Bd. Canada 24: 1837–1841.

JONES, N. R., AND J. MURRAY. 1966. Nicotinamide adenine dinucleotide (NAD) and reduced NAD in living and chill-stored dying muscle of cod, *Gadus callarias*. Bull. Japan. Soc. Sci. Fish. 32: 197–203.

KAPLAN, N. O. 1960. The pyridine coenzymes, p. 105–169. *In* P. D. Boyer, H. Lardy, and K. Myrbäck [ed.] The enzymes, Vol. 3. Academic Press, Inc., New York.

RAFTER, G. W., AND S. P. COLOWICK. 1957. Enzymatic preparation of DPNH and TPNH, p. 887–890. *In* S. P. Colowick and N. O. Kaplan [ed.] Methods in enzymology, Vol. III. Academic Press, Inc., New York.

TOMLINSON, N., E. S. ARNOLD, E. ROBERTS, AND S. E. GEIGER. 1961. Observations on *post mortem* biochemical changes in fish muscle in relation to *rigor mortis*. J. Fish. Res. Bd. Canada 18: 321–336.

WITTFOGEL, H. 1967. The use of ultra-violet light for determining whether fresh or frozen fish has passed through rigor mortis. FAO Technical Conference on the Freezing and Irradiation of Fish — Madrid. Topic III, No. FE: TFI/67/0/17.

Extensions of the Known Northern Range Limits of Ocean Whitefish, *Caulolatilus princeps*, and California Halibut, *Paralichthys californicus*

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The known northern range limit of *Caulolatilus princeps* Jenyns is extended some 120 nautical miles further north and that of *Paralichthys californicus* Ayres nearly 100 miles further northwest.

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Caulolatilus princeps — Ocean whitefish, *Caulolatilus princeps*, are known to occur along the California coast from Monterey southward to the Galapagos Islands, Ecuador (Jordan and Evermann, 1898). This species is not common north of Point Conception, California (Roedel, 1953). The present known northern range limit as reported by Robinson (1965) is off Willapa Bay, Washington (46°32′N to 46°43′N). A 42-cm ocean whitefish captured off the west coast of Vancouver Island, Canada, represents an extension of this range limit nearly 120 nautical miles further north. This fish was captured offshore between Barkley and Clayoquot Sounds, in 30–210 fath (48°41′N to 49°07′N) between January 18 and 27, 1965. The male specimen was landed by Mr Gunnar Ildhuso, master of the trawler *Georgene*. The fish is deposited in the College of Fisheries fish collection, University of Washington, No. U.W. 19830.

Paralichthys californicus — The range of the California halibut *Paralichthys californicus* was documented by Roedel (1953) as Central California south into the Gulf of California. Gunderson (1960) extended the range to 4–6 miles north of Redding Rock, California (41°20.5′N, 124°10.5′W). Magill (1962) extended the range to Long Beach, Washington (46°24′N, 124°15′W).

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On April 14, 1968, Michael Criscuola, master of the trawler *Lemes II*, captured a female specimen of ***Paralichthys californicus*** off the Quillayute River (47°55′N, 124°46′W) in 28–30 fath. The 86-cm fish captured off the Quillayute River represents a range extension of 100 miles northwest of that recorded by Magill (1962). The specimen was deposited in the College of Fisheries fish collection, University of Washington, No. 200318.

Acknowledgments — Our thanks to Richard B. Grinols of the U.S. Bureau of Commercial Fisheries, Seattle, Wash., for helpful suggestions in the preparation of this note.

REFERENCES

GUNDERSON, E. G. 1960. A range extension of the California halibut (*Paralichthys californicus*). Calif. Fish Game 46: 373–374.

JORDAN, D. S., AND B. W. EVERMANN. 1898. The fishes of north and middle America. Pt. III. U.S. Natl. Museum Bull. 47: 2274–2277.

MAGILL, AUSTIN R. 1962. Northerly range extension of the California halibut. Copeia 1962: 840–841.

ROBINSON, JACK G. 1965. New northern record for ocean whitefish. Oregon Fish. Comm. Res. Brief 11 p. 52.

ROEDEL, PHIL M. 1953. Common ocean fishes of the California coast. Calif. Fish Game Bull. 91: 184 p.

Fish School Size and Migration

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When a group of fish is migrating as a school, the error in navigation should be smaller as the size of the school increases, if the individuals jointly orient to a group mean direction. An approximation of this effect is available from consideration of the properties of the circular normal distribution.

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INDIVIDUAL FISH are capable of orientation that enables migration toward a goal (Hasler, 1967). When a group of fish is migrating as a school there are opportunities for the errors of individuals to be compensated, so that as the size of school increases, the error in navigation for the school will be lessened. Evaluation of this effect is discussed in this note.

The statistical properties of circular distributions that are involved in the summation of vectors are summarized by Watson and Williams (1956). For the question of accuracy of navigation the relevant probability density function is that which describes the distribution of angular deviations from the true direction at various sample sizes (N). If c is the cosine of the angle (θ) between the resultant vector (R) and the polar vector (or true direction), then the distance travelled in the true direction (X) is equal to Rc. When the individual vectors have circular normal distribution with concentration parameter $\varkappa$, the joint density of R and c is given by:

$$\frac{e^{\varkappa Rc}}{[I_0(\varkappa)]^N} \quad R\left\{\int_0^\infty [J_0(t)]^N J_0(Rt)\, t\,dt\right\} dR \frac{dc}{\pi(1-c^2)^{\frac{1}{2}}}$$

where $I_0(\varkappa)$, $J_0(t)$ and $J_0(Rt)$ are Bessel functions. Evaluation of this function would enable assessment of the dependence of c on N.

The form of this dependence may be approximated. (1) If the degree of direction-finding ability of individual fish is high, then the distribution of the directions taken might conform to the circular normal distribution. (2) If the individual fish show some directionality but the degree of direction finding is weak, or if the distribution is not circular normal, it will nevertheless be the case that we may consider groups of fish of size n, where n is large enough for the desired direction of the group to have a circular normal distribution.

In either case (1) or (2), the approximation (21) of Watson and Williams (1956) enables assessment of the relation between the error in the navigation and the size of the school (N individuals or N groups of size n), if $\varkappa$ is sufficiently large that the distribution of vectors is circular normal, and the bulk of the distribution falls within 90° of the true direction. From Watson and Williams:

$$2\varkappa(N-X) = \chi^2_{(p-1)\,(N)}$$

$$2\varkappa(N-R) = \chi^2_{(p-1)\,(N-1)}$$

where p is the number of dimensions (2 in the case of a circular distribution). Rearranging for $c = \frac{X}{R}$ we have

$$c = \frac{X}{R} = 1 - \frac{\chi^2_1}{2\varkappa N - \chi^2_{(N-1)}} \cdot \qquad (1).$$

Expressing the angular deviation (θ) in radians, $c = \cos\delta$, then if N is large, c will be approximated by the first two terms of the series

$$\cos\delta = 1 - \frac{\delta^2}{2\,!} + \frac{\delta^4}{4\,!} - \frac{\delta^6}{6\,!} + \ldots\ldots$$

Substituting $1 - \frac{\delta^2}{2}$ for c in equation 1, then

$$\delta^2 = \frac{2\chi_1^2}{2\kappa N - \chi^2_{(N-1)}} \,. \qquad (2).$$

The denominator of equation 2 is of order N, so that δ is proportional to $1/\sqrt{N}$, and it is apparent that the larger the size of school, the less is the error in joint navigation, provided κ is large and the denominator positive.

The effect of even a modest increase in school size is quite striking. For a circular normal distribution in which $\kappa = 2$, 92.5% of the deviates lie within 90° of the polar direction (Gumbel, et al., 1953). For this distribution the 95% limits of angular deviation for a school size of 5 are ±48.6°; for a school of 30 the deviation is ±18.3°; and for a school of 100 is ±9.5°. For $\kappa = 4$, comparable 95% limits for schools of 5, 30, and 100 are respectively ±28.6°; ±11.4°; and ±6.1°.

It follows from the above that, to achieve equal performance in migration, individual (solitary) fish would require more precise abilities for orientation than schooling fish that are jointly orienting to a group mean direction. The absolute values in the relation would be related to the distance to be traversed, the local environmental cues that can be exploited, and of course, other relevant considerations in school size such as predation.

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REFERENCES

GUMBEL, E. J., J. ARTHUR GREENWOOD, AND DAVID DURAND. 1953. The circular normal distribution: theory and tables. J. Am. Statist. Assoc. 48: 131–152.

HASLER, ARTHUR D. 1967. Underwater guideposts for migrating fishes, p. 1–15. *In* Animal orientation and navigation. Oregon State University Press, Corvallis, Ore.

WATSON, G. S., AND E. J. WILLIAMS. 1956. On the construction of significance tests on the circle and the sphere. Biometrika 43: 344–352.

First Massachusetts Bay Record of the Arctic Shanny *Stichaeus punctatus*

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The second Gulf of Maine specimen of the amphiboreal northern blenny *Stichaeus punctatus* is reported. This record extends the known southern range from Mount Desert, Maine, to Nahant in Massachusetts Bay. Ecological and morphological data on the specimen are also presented.

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TAYLOR ET AL. (1957, p. 335) noted the paucity of observations on southern ranges of northern forms in New England coastal waters compared with the number of northern records of southern species. This note extends the known southern range of a northern species, the arctic shanny, *Stichaeus punctatus* (Fabricius), 180 miles southwest from Mount Desert, Maine, to Nahant in Massachusetts Bay. *Stichaeus punctatus* is an amphiboreal northern blenny known from the Bering, Okhotsk, and Japanese seas in the Pacific and from Hudson Bay, western Greenland, Labrador, Newfoundland, and Nova Scotia in the Atlantic Ocean (Leim and Scott, 1966). There is one previous record from the Gulf of Maine — from the stomach of a cod taken off Little Duck Island, Mount Desert, Maine, on April 30, 1930 (Schroeder, 1931, Museum of Comparative Zoology 34576). In addition to the range extension, we also present morphological and ecological data.

The specimen (U.S. National Museum 203405) is a juvenile, 47.5 mm standard length, collected at the Northeastern University Marine Science Institute on East Point, Nahant, on August 22, 1968 by use of rotenone and scuba. It agrees well with descriptions in the literature (Bigelow and Schroeder, 1953; Makushok, 1958, 1961; Leim and Scott, 1966) and with specimens from Labrador, Greenland, Alaska, and the Kamchatka Peninsula (a total of 20 specimens in the USNM collections plus MCZ 34576 from Maine).

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The single lateral line extends posteriorly about half the length of the dorsal fin to the 25th dorsal spine on the left side and to the 19th spine on the right. The lateral line has 65 pores on the left and 50 on the right. The lateralis system on the head agrees with the specimen figured by Makushok (1958, fig. 51A) with a few minor exceptions: a small additional pore in the anterior part of the supraorbital canals near the anterodorsal margin of the orbits, an additional median pore in the fused posterior part of the supraorbital canal, two fewer pores in the postorbital part of the supraorbital canal on the right side, and two fewer pores in the canal leading posteriorly toward the dorsal fin origin from the supratemporal canal.

Shortly after preservation, the pigmentation was a mottled brownish except for the venter and the pelvic fins, which were immaculate white. The dorsal fin has five spots between spines 6–8, 17–18, 26–28, 37–40, and 45–47. In the fresh specimen, the posterior four spots consist of an anteroventral concentration of melanophores around an orange-red ocellus. The first spot has very little orange-red pigment. The suborbital bars are not as prominent as in the adult figured by Bigelow and Schroeder (1953) and Leim and Scott (1966). A few orange-red xanthophores are mixed in with the melanophores of the most prominent suborbital bar, which is the most posterior bar in the Nahant specimen.

Fin ray counts of the two Gulf of Maine specimens are dorsal XLIX; anal II,34 (Nahant), II,35 (Maine); pectoral 15–16 (Nahant), 15–15 (Maine); pelvic I,4. Bigelow and Schroeder (1953, p. 498) and Leim and Scott (1966, p. 313) reported only one anal spine. There may be either one or two anal spines, however (Jensen, 1944; Makushok, 1958). Fourteen of the 21 specimens we examined (including both Gulf of Maine specimens) and 9 of 17 examined by Jensen (1944, p. 37) had two anal spines. The two anal spines are attached to the first two pterygiophores as figured by Makushok (1958, fig. 52A). Vertebrae numbered 15 precaudal plus 39 caudal, total 54 in both Gulf of Maine specimens. The vertebrae in the other specimens examined numbered 15–16 + 38–41 = 53–56 compared with Makushok's (1958, p. 120) counts of 14–16 + 36–40 = 51–55.

The poison station was in a small rocky cove near the Marine Science Institute at low tide on the afternoon of August 22, 1968. One quart of rotenone (pro-nox) was poured around the base of rocks in water 5–10 ft deep. The water temperature was 56 F at 10 ft. Five other species of fishes were poisoned: *Tautogolabrus adspersus* (Walbaum), 8 specimens (49–97 mm standard length); *Myoxocephalus aeneus* (Mitchill), 3 (72–80); *Pholis gunnellus* (Linnaeus), 7 (110–130); *Liparis atlanticus* (Jordan and Evermann), 2 (30–32); and *Cyclopterus lumpus* Linnaeus, 1 (35). Other fishes commonly seen near this site include: *Pollachius virens* (Linnaeus), *Pseudopleuronectes americanus* (Walbaum), and *Ulvaria subbifurcata* (Storer).

Several species of seaweeds were abundant in the cove. *Ascophyllum nodosum* was found on the top rocks covered at high tide. *Polysiphonia lanosa* was epiphytic on the lower *A. nodosum*. *Chondrus crispus* was ubiquitous along the base of the rocky sides of the cove and extended 1½ ft up the sides. *Chondrus crispus* and *Enteromorpha linza* were on a large rock in the middle of the cove, and *E. linza* was also on a number of medium-sized stones. *Laminaria agardhii* was attached to stones at the base of the sides of the cove. There was also a 25 ft^2 patch of algae identified as *Chorda filum* at the mouth of the cove on August 22, but this patch was no longer present on a subsequent dive on October 4.

The stomach of the specimen was filled with recently ingested food, most likely poisoned organisms: 22 amphipods (3 *Jassa marmorata*, 18 *Calliopius laeviusculus*, 1 orchestid); 3 ostracods; 1 juvenile prosobranch gastropod; 6 juvenile mytillid pelecypods; 1 cumacean (*Diastylis polita*); and 1 zoea larval decapod.

A number of poison stations were made at East Point in late summer of 1967 and 1968, but only one specimen of *Stichaeus punctatus* was collected. Whether it is a southern stray or part of a small resident population remains to be determined.

Acknowledgments — We thank Mrs M. Dick for loaning us the MCZ specimen; Drs N. W. Riser, and M. P. Morse for identifying the stomach contents; and Drs V. G. Springer, S. A. Earle, and D. M. Cohen for reading the manuscript.

REFERENCES

BIGELOW, HENRY B., AND WILLIAM C. SCHROEDER. 1953. Fishes of the Gulf of Maine. U.S. Fish Wildlife Serv., Fish. Bull. 53: 1–577.

JENSEN, AD. S. 1944. Contributions to the ichthyofauna of Greenland. 6. The Greenland species of the genera *Stichaeus* and *Eumesogrammus* (Teleostei, Percomorphi — Stichaeidae). Spolia Zool. Museo Haun. 4: 36–39.

LEIM, A. H., AND W. B. SCOTT. 1966. Fishes of the Atlantic coast of Canada. Bull. Fish. Res. Bd. Canada 155. 485 p.

MAKUSHOK, V. M. 1958. [The morphology and classification of the northern blennioid fishes (Stichaeoidae, Blennioidei, Pisces).] Tr. Zool. Inst. Akad. Nauk SSSR 25: 3–129 (In Russian, Bur. Com. Fish. Systematics Lab. Transl. 1.)

1961. [Some peculiarities in the structure of the seismosensory system of the northern blenniids (Stichaeoidae, Blennioidei, Pisces).] Tr. Inst. Okeanol. 43: 225–269 (In Russian, Bur. Com. Fish. Systematics Lab. Transl. 19.)

SCHROEDER, WILLIAM C. 1931. Notes on certain fishes collected off the New England coast from 1924 to 1930. Boston Soc. Nat. Hist. Bull. 58: 3–8.

TAYLOR, CLYDE C., HENRY B. BIGELOW, AND HERBERT W. GRAHAM. 1957. Climatic trends and the distribution of marine animals in New England. U.S. Fish Wildlife Serv., Fish. Bull. 57: 293–345.

Use of Spinner Culture Cells to Detect Water Pollution

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Li, M. F., and Carol Jordan. 1969. Use of spinner culture cells to detect water pollution. J. Fish. Res. Bd. Canada 26: 1378–1382.

The sensitivity of L-cells (spinner culture) to several pesticides and a herbicide was determined. The inhibition of L-cells growth was marked and in the case of DDT was shown to be strictly concentration-dependent. The simplicity and reproducibility of the assay system should make it a useful tool in water pollution studies.

Received October 24, 1968

Pesticides and detergents are among the most destructive types of pollutants to which populations of fish and shellfish are exposed (Sprague, 1967). Fish have been employed as biological indicators in bioassays of water pollutants (Lennon and Walker, 1964), but this technique is both laborious and time consuming. Furthermore the results obtained from independent bioassays with different fish often cannot be correlated (Lennon, 1967) since the resistance of the fish to pollutants varies from one species to another (Henderson, 1960). Even within a species the size, age, and physiological condition of the fish would affect its resistance to a particular pollutant.

Mammalian cells cultivated in vitro are sensitive to certain pesticides present in the growth medium (Gabliks and Friedman, 1965; Gabliks, 1965a, b; Wilson and Walker, 1966; Gabliks et al., 1967). We have explored the possibility of using the spinner culture technique for the rapid detection of water pollution. The present communication describes the inhibition of growth of cells in spinner culture by a number of pesticides and a herbicide.

Cell culture technique — The cell line, arising from clone 929[1] (Earl's L-cells; mouse fibroblast) used in the bioassays was grown in 50-ml spinner culture apparatus[2] at 37 C. Mixing at 200 rpm was accomplished by motorized magnets[3] located under the spinner and outside the constant temperature water bath (Fig. 1). This simple arrangement permitted the growth of spinner cultures while eliminating the need for elaborate incubation facilities. Spinner medium (MEM with Hanks' base),[4] supplemented with 10% fetal calf serum was used as the growth medium. Methylcellulose and Darven[5] were added at concentrations of 1.2% and 0.5%, respectively, to prevent cell clumping and cell damage resulting from the agitation (Merchant and Hellman, 1962). Penicillin (50 units/ml), streptomycin (50 units/ml), and mycostatin (25 units/ml) were added to the growth medium to inhibit microbial growth.

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[1] Microbiological Associates, Bethesda, Maryland.

[2] Bellco Glass Inc., Vineland, New Jersey.

[3] Hurst synchronous motor, Type CA-200, Radioshack Corp., Boston, Mass.

[4] Becton, Dickinson and Co. Canada Ltd., Clarkson, Ont.

[5] Methocel 15 cps, reagent grade, Dow Chemical Co., Midland, Michigan; Darven No. 2, R. T. Vanderbilt Co. Inc., New York 17, N.Y.

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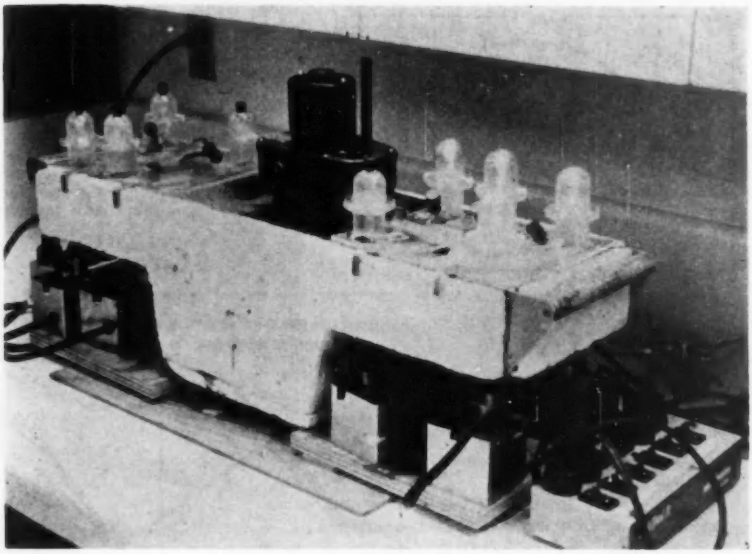

FIG. 1. Spinner culture apparatus.

Pesticides and herbicide — The Malathion, DDT, Lindane, and 2-4-D Ester were obtained from commercial sources.[6] The concentration of each agent was calculated from its listed active component(s) and expressed as parts per million (ppm) of the experimental cultures.

Growth assay — The pesticides and the herbicide were dissolved or suspended at 10 times the desired concentration in Hanks' balanced salt solution. Since steam sterilization did not affect the toxicity of these agents for the cells (unpublished data) the prepared solutions or suspensions were autoclaved at 15 psi for 15 min. Three ml of Hanks' balanced salt solution containing a pesticide or the herbicide or neither were mixed with 27 ml of the growth medium to which cells (in the active growth phase) were added from the stock spinner culture. The initial cell density of the experimental cultures ranged from 1.5×10^5 to 2.5×10^5 per ml. The cell counts were taken with an electronic cell counter as previously described (Li and Stewart, 1965). Unless otherwise indicated each assay was terminated at the end of the 3rd day.

A typical normal growth curve of the cells is shown in Fig. 2. A 3-day experimental period was appropriate for the present study. Growth of the cells was inhibited by Malathion and DDT (25 and 10 ppm), and Lindane and 2-4-D Ester (10 and 5 ppm) (Fig. 3). The effect of these agents on cell growth is summarized in Table 1.

Increasing inhibition of the cell growth coincided with increasing DDT concentration in the growth medium. The concentrations of the pesticide

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[6] Malathion 50, Niagara Brand Chemicals, Burlington, Ont.; Ortho Best-B-Gon 50% DDT, Ortho Agricultural Chemical Ltd., Oakville, Ont.; Lindane, 25% wettable powder, Green Cross Products; 2-4-D Liquid Weed Killer, Green Cross Products, The Sherwin-Williams Co. of Canada Ltd., Montreal.

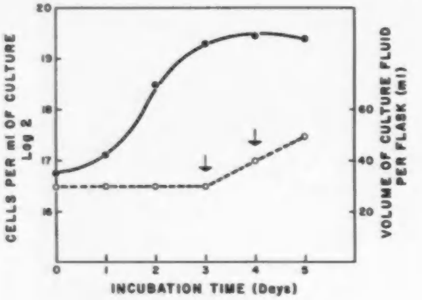

FIG. 2. Growth pattern of L-929 mouse cells in the stock spinner culture at 37 C. ● —— ● indicates number of cells per ml; ○ — — — ○ indicates total cumulative volume of culture fluid. Arrows indicate time of addition of growth medium.

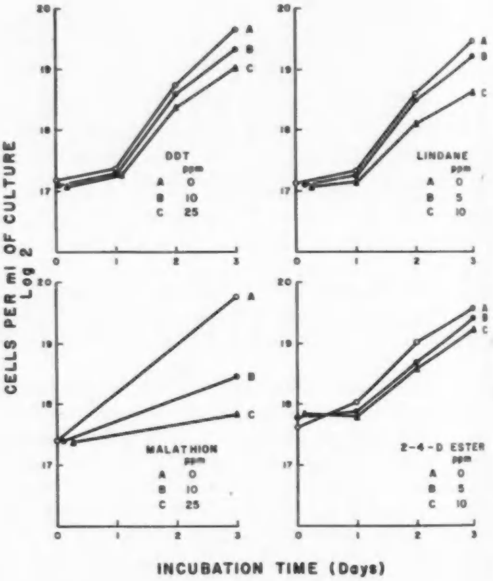

FIG. 3. Effect of DDT, Malathion, Lindane, and 2-4-D Ester on growth of the L-cells at 37 C. Each point represents the mean value of two determinations (for DDT, Malathion, and Lindane) or one determination (for 2-4-D Ester).

were plotted against the percentage of growth inhibition (Fig. 4) and a regression analysis was carried out. The correlation coefficient for the comparison of the concentration of the pesticide with the percentage of the growth inhibition was 0.91 indicating that the regression was valid at the 1% level.

The initial cell density of the culture affected inhibition of growth by the pesticide. An increase in the initial cell density from 1.6×10^5 to 3.2×10^5 per ml reduced the inhibition by 50 ppm of DDT from a value of approximately 50% to a value of approximately 16% (Table 2).

The cells propagated in the spinners exhibited a short lag phase and a mean generation time of 28 hr in the 3-day incubation period. This lies in the upper range of generation times observed for L-cells by other workers

TABLE 1. Effect of various pesticides and a herbicide on the cell growth for 3 days at 37 C.

Pesticides and herbicide	% inhibition		
	25 ppm	10 ppm	5 ppm
Malathion[a]	72.0	61.4	
DDT[a]	24.7	12.9	
Lindane[a]		34.2	12.4
2-4-D Ester[b]		31.6	17.7

[a] Mean value of two determinations.
[b] Value of one determination.

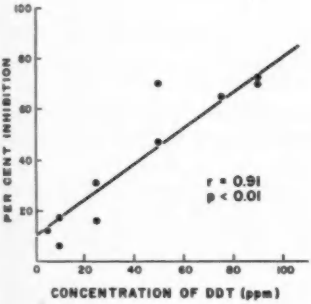

FIG. 4. The relationship of DDT concentration to per cent inhibition of cell growth in spinner culture for 3 days at 37 C.

TABLE 2. Effect of initial cell density on the inhibition of cell growth by DDT (50 ppm).

Initial cell density (*cell/ml*)	% inhibition	
	Replicate 1	Replicate 2
1.6×10^5	48.6	51.4
3.2×10^5	15.2	17.4

(McLimans et al., 1957). The spinner culture technique not only permits a luxuriant growth of the cells but also allows a study of continuous cell growth to be conducted without sacrificing the culture. An additional advantage in the use of spinner cultures lies in a more reliable assessment of cell numbers in contrast to replicate stationary cultures where errors inevitably occur through the loss of unattached cells (Wilson and Walker, 1966). The degree of growth inhibition of the cells by DDT correlated well with the concentration of the pesticide. The initial cell density and possibly other yet unrecognized factor(s) may significantly affect the per cent growth inhibition by pesticides; for better results positive controls should be run with specimens. Although our cell system was quite sensitive to Malathion (Gabliks and Friedman, 1965; Wilson and Walker, 1966; Gabliks et al., 1967), the sensitivity of the present assay system to this and other pollutants can probably be further improved by selection of an optimal initial cell density and use of a simpler medium. Further work should include a comparison of the results using this assay method with bioassays using live fish and shellfish. The simplicity of the method should make it a useful tool in screening water samples for pollutants.

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REFERENCES

Gabliks, J. 1965a. Responses of cell cultures to insecticides. II. Chronic toxicity and induced resistance. Proc. Soc. Exptl. Biol. Med. 120: 168–171.

1965b. Responses of cell cultures to insecticides. III. Altered susceptibility to Poliovirus and Diphtheria Toxin. Proc. Soc. Exptl. Biol. Med. 120: 172–175.

Gabliks, J., Myrna Bantug-Jurrila, and L. Friedman. 1967. Responses of cell cultures to insecticides. IV. Relative toxicity of several organophosphates in mouse cell cultures. Proc. Soc. Exptl. Biol. Med. 125: 1002–1005.

Gabliks, J., and L. Friedman. 1965. Responses of cell cultures to insecticides. I. Acute toxicity to human cells. Proc. Soc. Exptl. Biol. Med. 120: 163–168.

Henderson, C. 1960. Bioassay procedures, aims and equipment. U.S. Dept. Health, Educ. Welfare, Public Health Serv. Tech. Rept. W60-3: 246–248.

Lennon, R. E. 1967. Selected strains of fish as bioassay animals. Progressive Fish-Culturist 29: 129–132.

Lennon, R. E., and C. R. Walker. 1964. Investigations in fish control: 1. Laboratories and methods for screening fish-control chemicals. Bur. Sport Fish. Wildlife Circ. 185. 15 p.

Li, M. F., and J. E. Stewart. 1965. A quantitative study of the effects of naturally occurring supplements on the growth of rainbow trout (*Salmo gairdneri*) gonadal cells. Canadian J. Microbiol. 11: 9–14.

McLimans, W. F., E. V. Davis, F. L. Glover, and G. W. Rake. 1957. The submerged culture of mammalian cells: the spinner culture. J. Immunol. 79: 428–433.

Merchant, D. J., and K. B. Hellman. 1962. Growth of L-M strain mouse cells in a chemically defined medium. Proc. Soc. Exptl. Biol. Med. 110: 194–198.

Sprague, J. B. 1967. Impact of water pollution on fisheries in the Atlantic provinces. Canadian Fish. Rept. 9: 11–15.

Wilson, B. W., and N. E. Walker. 1966. Toxicity of Malathion and Mucaptosuccinate to growth of chick embryo cells *in vitro*. Proc. Soc. Exptl. Biol. Med. 121: 1260–1264.

Swimming Thrust of Sockeye Salmon (*Oncorhynchus nerka*) in Relation to Selectivity of Gillnets

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LANDER, R. H. 1969. Swimming thrust of sockeye salmon (*Oncorhynchus nerka*) in relation to selectivity of gillnets. J. Fish. Res. Bd. Canada 26: 1383–1385.

A significant relationship between girth at the mesh mark and fork length indicates that long sockeye salmon (*Oncorhynchus nerka*) have more swimming thrust than short ones. This tends to confirm a speculation of Regier and Robson (J. Fish. Res. Bd. Canada 23: 423–454, 1966). Preliminary conjecture relates the observation to the theory of mesh selectivity.

Received November 13, 1968

THE PRESENT NOTE touches on the relation of girth at the mesh mark to fish length in terms of swimming thrust. The fish were maturing sockeye salmon caught on the high seas; they were a subsample of those for which the relations of maximum girth to fork length were reported earlier (Lander, 1963).

Regier and Robson (1966) intensively reviewed and considerably extended the theory for estimating selectivity curves for gillnets. The authors noted that McCombie and Fry (1960) considered girth to be the best measure of fish size, but speculated (page 435) "...the swimming thrust that the fish can exert may be more important and this thrust may be more closely correlated with length than with girth."

Dr R. B. Thompson and his colleagues (personal communication) at our laboratory observed in experimental ponds that sockeye salmon struggled in the nets to exhaustion, then rested, whereas pink salmon (*Oncorhynchus gorbuscha*) fought the nets less vigorously but rested very little. Hence behavioral differences between morphologically similar species should play a role in selectivity.

Four aspects of selectivity in gillnets are well known for smooth, fusiform fishes: (1) large fish are wedged more anteriorly than small fish in a given mesh size; (2) fish of a given size are wedged more anteriorly in small than in large meshes; (3) fish with head girths larger than the mesh lumen cannot enter but may be snagged by the teeth or opercle; and (4) fish with maximum girths smaller than the mesh lumen typically escape but may be entangled in the webbing. The foregoing generalizations appear justified by the studies of Holt (1963), McCombie and Fry (1960), Japanese investigations (e.g., Taguchi, 1961; Ueno et al., 1965), and by experience and judgements of the present author and Dr H. A. Regier (personal communication, December 1968).

The role of length is limited in present methods (e.g., the indirect and iterative methods in Regier and Robson, 1966) to description of retention probabilities in terms of fork or total length. If girth at the mesh mark is independent of fish length, then long fish of vulnerable size evidently penetrate to an absolute girth no greater than that of short fish before wedging; in that situation, swimming thrust may not influence retention probabilities within the limits of head and maximum girth (or, therefore, within the approximate limits of the selectivity curve for the mesh size in question). If, on the other hand, girth at the mesh mark increases with fish length, then long fish penetrate to a greater absolute girth before wedging and evidently have more swimming thrust than do short fish. Joint measures of length and girth at various body sites between the snout and the point of maximum girth might then be combined into a functional parameter.

Girth approaches maximum girth asymptotically as a function of the distance from tip of snout to the point of maximum girth; an analogy in growth studies is the curve of natural logarithm of weight on age (e.g., Ricker, 1958).

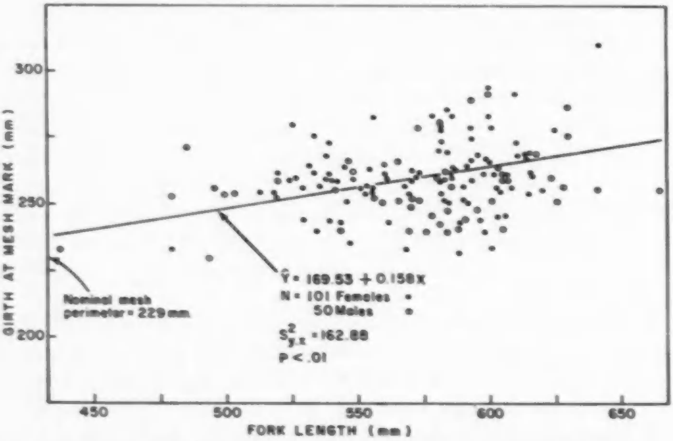

FIG. 1. Relation of girth at mesh mark to fork length of maturing sockeye salmon caught 30–300 miles south of the Shumagin Islands, Alaska, during June 1961 in 4½-inch (114.3-mm) gillnets.

A size specific range of meshing sites on the body may characterize the large-sample frequency of meshing-girth: maximum-girth ratios. These ratios might be related in turn to mesh perimeter and hence retention probabilities; i.e., the closer the mesh mark to the point of maximum girth, the lower the chance of retention. The ratios would increase with fish length if large fish have more swimming thrust than small ones. These rudimentary notions may or may not warrant further study.

In any case, the present data indicate that girth at the mesh mark increased with fork length (Fig. 1). Long sockeye salmon apparently had more swimming thrust than short ones in this situation. The interpretation assumes that the compressibility of large fish did not exceed that of small fish; the interpretation is strengthened if actual compressibility was lower for large fish.

REFERENCES

Holt, S. J. 1963. A method of determining gear selectivity and its application. Intern Comm. Northwest Atlantic Fish. Spec. Publ. 5: 106–115.

Lander, Robert H. 1963. Girth–length relationships in sockeye and chum salmon. Trans. Am. Fish. Soc. 92: 305–307.

McCombie, A. M., and F. E. J. Fry. 1960. Selectivity of gill nets for lake whitefish, *Coregonus clupeaformis*. Trans. Am. Fish. Soc. 89: 176–184.

Regier, H. A., and D. S. Robson. 1966. Selectivity of gill nets, especially to lake whitefish. J. Fish. Res. Bd. Canada 23: 423–454.

Ricker, W. E. 1958. Handbook of computations for biological statistics of fish populations. Bull. Fish. Res. Bd. Canada 119. 300 p.

Taguchi, Kisaburo. 1961. On the suitable mesh size on salmon gillnets inferred from the relationship between body weight of salmon caught by gillnets and its mesh size. Bull. Japan Soc. Sci. Fish. 27: 645–649. (English abstr.)

Ueno, Motokazu, Seikichi Mishima, and Kenji Shimakazi. 1965. On the falling and escaping of the salmon from the gill net. Bull. Fac. Fish. Hokkaido Univ. 40: 71–77. (English abstr.)

Characteristics of *Gaffkya homari*, the Causative Agent of the Lobster Disease Gaffkemia

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Stewart, James E., Diane M. Foley, and R. G. Ackman. 1969. Characteristics of *Gaffkya homari*, the causative agent of the lobster disease gaffkemia. J. Fish. Res. Bd. Canada 26: 1385–1389.

Gaffkya homari did not exhibit lipase, protease, or lecithinase exoenzyme activities. The organism was coagulase and fibrinolysin negative; only lactic acid was produced from glucose. The capacity of whole lobster hemolymph or its components to promote the growth of *G. homari* was assessed.

Received October 22, 1968

Gaffkya homari is the causative agent of gaffkemia, a fatal disease of lobsters (*Homarus americanus* and *H. vulgaris*) (Snieszko and Taylor, 1947; Hitchner and Snieszko, 1947; Roskam, 1957). Our work on this disease (Stewart et al., 1966a; Cornick and Stewart, 1968a, b) and the work of Rabin and Hughes (1968) have shown that lobster hemolymph forms an excellent medium for the growth of this organism. Rabin (1965) has shown that in gaffkemia *G. homari* is confined to the hemolymph. It has not been shown, however, which

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(features) of this bacterium is the cause of death during infection. We have undertaken an in vitro study of this organism to obtain information on which to base in vivo studies to determine the actual cause of death.

Enzymes — Tests for lipase, protease, and lecithinase production using *G. homari* ATCC 10400 and *Gaffkya tetragena* ATCC 10875 strains were made using methods cited in the Manual of microbiological methods (Society of American Bacteriologists, 1957). A test for fibrinolytic activity was made by stab and streak inoculation of tubes of sterile, clotted (nonretracting) lobster hemolymph with these organisms. *Gaffkya homari* ATCC 10400 was tested for coagulase activity with Bacto-coagulase plasma and with whole lobster plasma (prepared without anticoagulants; Stewart et al., 1966b). Protein concentration was determined by Robinson and Hogden's modification of the biuret method (Layne, 1957); bovine serum albumin (Pro Sol[1]) was used as the standard.

Neither *G. homari* nor *G. tetragena* hydrolysed casein, gelatin, tributyrin, or butterfat and neither produced a lecithinase type of reaction in agar or broth-based egg yolk medium. Neither organism possessed a fibrinolysin effective against clotted lobster hemolymph although they both grew well at 28 C on the surface of the clot and along the line of the stab. They both produced a white zone in the clotted hemolymph in the immediate area of the growth. Presumably, this was the effect of the bacterially produced lactic acid on the hemocyanin. *Gaffkya homari* ATCC 10400, the only strain tested, was coagulase negative in Bacto-coagulase plasma and whole lobster plasma.

Growth product — *Gaffkya homari* ATCC 10400 and *G. tetragena* ATCC 10875 were grown at 28 C in glucose–yeast extract–tryptone–phosphate (GYTP) broth (Skerman, 1959) until changes in the *p*H of the medium ceased. The cultures and the uninoculated controls were then clarified with $ZnSO_4$ (Neish, 1952). Samples of the clarified filtrates were injected directly on to a gas chromatographic polyester/phosphoric acid column employing a flame ionization detector. The presence of lactic acid was detected by gas–liquid chromatography of methyl esters prepared from constituents of the filtrates and by the quantitative chemical method of Barker and Summerson (1941). Glucose was determined by the anthrone method of Morris (1948).

The direct gas chromatographic analysis for growth products showed only traces of ethanol and lesser but equal amounts of methanol and propanol from both the inoculated and the uninoculated GYTP broth filtrates. Gas-liquid chromatography of methyl esters prepared from ether extracts of the acidified filtrates showed that lactic acid was the only growth product. The chemical determinations of glucose and lactic acid showed that the amount of lactic acid produced balanced the amount of glucose that had disappeared. *Gaffkya homari* produced 0.5% lactic acid, and *G. tetragena* 0.8% lactic acid (final concentrations in the culture filtrate).

Cytochrome — The method of Deibel and Evans (1960) was used for the detection of cytochrome in bacteria grown at 30 C on Difco[2] blood agar base without added blood. The reactions of several strains of *G. homari* (ATCC 10400, Rabin's strain, and three *G. homari* strains isolated by us from local lobsters), and *G. tetragena* ATCC 10875 were compared with the reactions given by one strain each of *Pseudomonas fluorescens* and *Micrococcus conglomeratus*. The pseudomonad gave the expected dark blue color, *M. conglomeratus* a medium blue, *G. tetragena* a very light blue color only at the edges and toward the end of the streaked line of growth possibly because of the presence of catalase; none of the five *G. homari* strains gave any reaction. The results for *G. homari* strains are consistent with their homofermentative production of lactic acid while growing in the presence of glucose.

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[1] Standard Scientific Supply Corporation, New York, N.Y.

[2] Difco Laboratories Inc., Detroit 1, Michigan.

Effect of lobster hemolymph on growth — At 28 C the growth of *G. homari* in lobster hemolymph serum or plasma in vitro is maximal in 24 hr; the total numbers of *G. homari* obtained varies directly with the dilution (Fig. 1). The considerable variation in the concentration of hemolymph components between individual lobsters (Stewart et al., 1967; Telford, 1965, 1968) and the rapid fluctuation in certain components (Telford, 1968) would affect the total numbers of *G. homari* obtained. This should be particularly noticeable when using serum samples taken from individual lobsters. Such differences in the growth of *G. homari* in individual lobster serum samples were observed by Rabin and Hughes (1968) in their unsuccessful attempt to correlate the growth of *G. homari* in individual serum samples with the susceptibility of the donor lobster to infection with this bacterium. The differences in growth in the individual serum samples noted by Rabin and Hughes (1968) could be a result of the variations and fluctuations in the concentration of key components in the hemolymph.

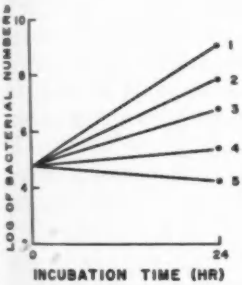

FIG. 1. Growth of *Gaffkya homari* in lobster hemolymph serum. Protein concentration used as the index of dilution: (1) undiluted serum 44 mg/ml; (2) 6.2 mg/ml; (3) 2.2 mg/ml; (4) 1.3 mg/ml; (5) 0 mg/ml. Dilutions made with 3% NaCl; incubation temperature, 28 C.

The comparatively slow growth of *G. homari* in the defined medium of Aaronson (1956) (5–7 days compared with 24 hr in lobster hemolymph) suggested that lobster hemolymph was supplying additional or different growth factors. The deletion and replacement type of experiment (Deibel et al., 1964) employing all purpose medium was used to assess the effect that the different components of lobster hemolymph had on the growth of *G. homari*.

The lobster hemolymph serum or plasma was prepared by methods reported earlier (Stewart et al., 1966b) and was sterilized by membrane filtration (0.45 mμ pore size) and added aseptically to the sterile, cooled all purpose medium broth or variations of it. The presence of either thiamine or Tween 80 (polyoxyethylene sorbitan monooleate) was slightly inhibitory to the growth of *G. homari*; consequently both were left out, the complete assay medium was all purpose medium minus thiamine and Tween 80. The inhibitory effect of Tween 80 indicates that *G. homari* does not have an oleate requirement (Deibel et al., 1964).

Maximal growth occurred in the complete assay medium in 24 hr at 28 C; a 27-hr growth period was chosen for assay purposes. A replacement of 30–50% of the complete assay medium with undialysed lobster serum resulted in an increase in total growth of 20–25%.

Deletion of the following from the assay medium caused a reduction in growth of approximately 25% for tryptone, 50% for yeast extract, and 90% for glucose. A summary of the data gained by replacing these constituents with lobster hemolymph serum or plasma or with an amino acid mixture indicated: (1) that an amino acid mixture equivalent to the free amino acids of lobster hemolymph (quantitatively and qualitatively) is an adequate nitrogen source, almost as good as tryptone and that any one of the amino acids was an adequate nitrogen source when yeast extract was present; (2) that the carbohydrate level in lobster hemolymph was the growth-limiting factor in vitro (a maintenance of the carbohydrate levels at the expense of other tissue would be expected in vivo); (3) that the hemolymph is supplying a nondialysable growth-stimulating factor almost equal to tryptone in growth-stimulating value (possibly a peptide, Jones and Woolley, 1962); and (4) that the hemolymph is supplying in addition to the nondialysable factor, dialysable growth factor(s) not provided by the medium of Aaronson (1956).

Gaffkya homari's lack of proteolytic, lipolytic, and fibrinolytic exoenzymes suggests that the harmful effects on lobsters infected with this organism do not result from direct destruction of the host tissue. The inability of *G. homari* to produce its own portal of entry (Wood, 1965; Rabin and Hughes, 1968; Stewart et al., 1969) is in complete agreement with the absence of these exoenzymes. The absence of coagulase activity suggests that the infection in the lobster would not be localized; this is consistent with the information supplied by Rabin (1965) and Cornick and Stewart (1968a) indicating that gaffkemia is septicemic in nature. None of the data here rule out the direct involvement of a toxin or secondary toxic effects as the cause(s) of death but no evidence of these effects has as yet been detected and the characteristics of *G. homari* suggest that gaffkemia would be, to a large extent, a wasting type of disease.

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REFERENCES

Aaronson, S. 1956. A biochemical-taxonomic study of a marine micrococcus, *Gaffkya homari*, and a terrestrial counterpart. J. Gen. Microbiol. 15: 478–484.

Barker, S. B., and W. H. Summerson. 1941. The colorimetric determination of lactic acid in biological material. J. Biol. Chem. 138: 535–554.

Cornick, J. W., and J. E. Stewart. 1968a. Interaction of the pathogen *Gaffkya homari* with natural defense mechanisms of *Homarus americanus*. J. Fish. Res. Bd. Canada 25: 695–709.

1968b. Pathogenicity of *Gaffkya homari* for the crab *Cancer irroratus*. J. Fish. Res. Bd. Canada 25: 795–799.

Deibel, R. H., and J. B. Evans. 1960. Modified benzidine test for the detection of cytochrome-containing respiratory systems in microorganisms. J. Bacteriol. 79: 356–360.

Deibel, R. H., J. H. Silliker, and P. T. Fagan. 1964. Some characteristics of an oleate-requiring hemolytic pediococcus. J. Bacteriol. 88: 1078–1083.

Hitchner, E. R., and S. F. Snieszko. 1947. A study of a microorganism causing a bacterial disease of lobsters. J. Bacteriol. 54: 48.

Jones, K. M., and D. W. Woolley. 1962. Peptides required for the growth of *Lactobacillus bulgaricus*. J. Bacteriol. 83: 797–801.

Layne, E. 1957. Spectrophotometric and turbidimetric methods for measuring proteins, p. 450. *In* S. P. Colowick and N. O. Kaplan [ed.] Methods in enzymology, Vol. 3. Academic Press, Inc., New York.

Morris, D. L. 1948. Quantitative determination of carbohydrates with Dreywood's anthrone reagent. Science 107: 254–255.

Neish, A. C. 1952. Analytical methods for bacterial fermentations. Natl. Res. Council Canada Rept. 46-8-3, 2nd revision, NRC 2952, Ottawa, Ont. 69 p.

Rabin, H. 1965. Studies on gaffkemia, a bacterial disease of the American lobster, *Homarus americanus* (Milne Edwards). J. Invertebrate Pathol. 7: 391–397.

Rabin, H., and J. T. Hughes. 1968. Studies on host–parasite relationships in gaffkemia. J. Invertebrate Pathol. 10: 335–344.

Roskam, R. Th. 1957. Gaffkaemia, a contagious disease in *Homarus vulgaris*. Conseil Perm. Intern. Exploration Mer Shellfish Comm. Charlottenlund Slot, Danemark. 4 p.

Skerman, V. B. D. 1959. A guide to the identification of the genera of bacteria. The Williams and Wilkins Co., Baltimore, Maryland. p. 178.

Snieszko, S. F., and C. C. Taylor. 1947. A bacterial disease of the lobster (*Homarus americanus*). Science 105: 500.

Society of American Bacteriologists. 1957. Manual of microbiological methods. McGraw-Hill Book Company, Inc., New York. 315 p.

Stewart, J. E., J. W. Cornick, D. M. Foley, M. F. Li, and C. M. Bishop. 1967. Muscle weight relationship to serum proteins, hemocytes, and hepatopancreas in the lobster, *Homarus americanus* J. Fish. Res. Bd. Canada 24: 2339–2354.

Stewart, J. E., J. W. Cornick, D. I. Spears, and D. W. McLeese. 1966a. Incidence of *Gaffkya homari* in natural lobster (*Homarus americanus*) populations of the Atlantic region of Canada. J. Fish. Res. Bd. Canada 23: 1325–1330.

Stewart, J. E., J. R. Dingle, and P. H. Odense. 1966b. Constituents of the hemolymph of the lobster, *Homarus americanus* Milne Edwards. Canadian J. Biochem. 44: 1447–1459.

Stewart, J. E., A. Dockrill, and J. W. Cornick. 1969. Effectiveness of the integument and gastric fluid as barriers against transmission of *Gaffkya homari* to the lobster *Homarus americanus*. J. Fish. Res. Bd. Canada 26: 1–14.

Telford, M. 1965. Carbohydrates in the blood of the lobster, *Homarus americanus*. Canadian J. Zool. 43: 503–507.

1968. The effects of stress on blood sugar composition of the lobster, *Homarus americanus*. Canadian J. Zool. 46: 819–826.

Wood, P. C. 1965. A preliminary note on gaffkaemia investigations in England. Rappt. Procès-Verbaux Réunions Conseil Perm. Intern. Exploration Mer 156: 30–34.

Distribution of Insular Fishes of Sable Island, Nova Scotia

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GARSIDE, E. T. 1969. Distribution of insular fishes of Sable Island, Nova Scotia. J. Fish. Res. Bd. Canada 26: 1390–1392.

Collections of fishes from the several bodies of fresh and brackish waters on Sable Island, Nova Scotia (60°06′W, 43°56′N), were composed variously of four euryhaline species: *Fundulus heteroclitus* (Cyprinodontidae), *Gasterosteus wheatlandi*, *Apeltes quadracus*, and *Pungitius pungitius* (Gasterosteidae). Sight records are reported for *Anguilla rostrata* (Anguillidae).

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A SURVEY OF the insular piscifauna of Sable Island, Nova Scotia, was conducted from July 2–5, 1968.

Sable Island is a relatively stable, crescentic bar of sand situated approximately 290 km east of Halifax, N.S. The length of the exposed arc is 39 km and the maximum width of the bar is approximately 1.5 km. The main station (West Light) is situated at 60°06′W, 43°56′N. The present form of the island and locations of fixed structures and basins of standing water are shown in Fig. 1. The southern shore, which had been breached at numerous points previously, became consolidated in the interval between 1766 and 1851, according to successional maps (Cameron, 1965), creating an elongate entrapment nearer to that shore. Subsequently, progressive reduction of the standing water has occurred leaving Lake Wallace as the most extensive and

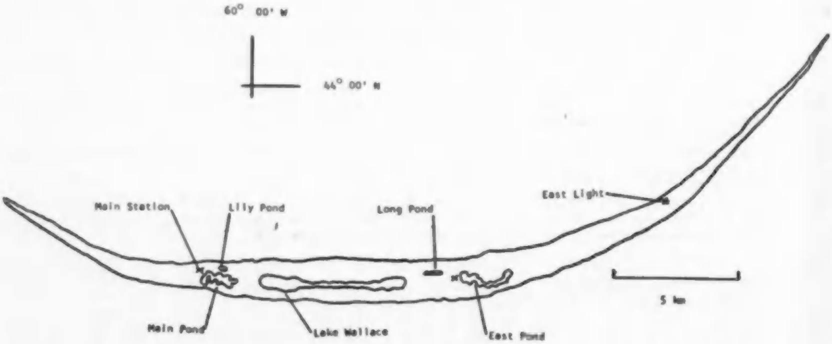

FIG. 1. Sable Island, N.S., showing principal sites of fixed structures and the standing waters that were sampled.

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strongly saline remanent. Four ponds of significant area are shown in Fig. 1 also. Three are quite fresh; the last is moderately brackish. I have applied names to these ponds for convenient reference (see Table 1).

Mummichog, ***Fundulus heteroclitus*** (L), (Cyprinodontidae), blackspotted stickleback, ***Gasterosteus wheatlandi*** Putnam, fourspine stickleback ***Apeltes quadracus*** (Mitchill), and ***Pungitius pungitius*** (L.), (Gasterosteidae), form the total composition of the collections. Distribution of these species is given in Table 1.

TABLE 1. Distribution of fishes in insular waters of Sable Island, N.S., July 1968. Temperature and salinity levels of surface water at the time of collections are given for each body of water.

	Species distribution			
Location	*Fundulus heteroclitus*	*Gasterosteus wheatlandi*	*Apeltes quadracus*	*Pungitius pungitius*
Lake Wallace (16 C, 26‰)	Nil	Nil	Nil	Nil
Main Pond (21 C, 0‰)	Present	"	"	Present
Lily Pond (21 C, 0‰)	Nil	"	"	Present
Long Pond (21 C, 0‰)	Nil	"	"	Nil
East Pond (21 C, 16‰)	Present	Present	Present	Present

Lake Wallace is reported to have a depth approaching 8 m. Cameron (1965) suggests that the salinity of Lake Wallace is maintained by wind-borne spray from the shore, approximately 200 m distant. However, a salinity of 26‰ at the surface of a body of this size suggests that this salinity is residual but has some dilution by freshwater drainage. The lake appears to support substantial populations of an assortment of crustaceans, and bivalve and gastropod molluscs. Although personnel of the Canada Department of Transport who maintain the meteorological station reported that "flounders" had been seen by divers 2 years previously, I was unable to collect any fish in this lake, using a fine-mesh gillnet, a hoop net, and a 10-m seine.

East Pond, which lies immediately east of the abandoned rescue station, probably is also a remanent of the original entrapment, which has been diluted subsequently by drainage from surrounding elevations. All species known to be in insular waters of Sable Island were collected in this pond. These fish are noted for their euryhaline tolerance.

Main Pond, Lily Pond, and Long Pond lie north of a long irregular ridge that separates the south shore, including the extensive beach containing

Lake Wallace, from the north side of the arc. The bodies are quite fresh, all having salinity values less than 0.05‰, and are maintained by drainage. These are probably relatively recently formed ponds and, where fish are present, they probably have been introduced fortuitously by birds, wind, or possibly the deliberate action of inhabitants.

An interesting sight record is that for the American eel, *Anguilla rostrata* (LeSueur), which has been seen in Main Pond on three occasions by Dr I. A. McLaren (personal communication). Presumably these individuals migrated overland or were transported by wind or birds.

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REFERENCES

CAMERON, H. L. 1965. The shifting sands of Sable Island. Geograph. Rev. 55: 463-476.

A Precipitin-like Reaction of the Hemolymph of the Lobster *Homarus americanus*

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STEWART, JAMES E., AND DIANE M. FOLEY. 1969. A precipitin-like reaction of the hemolymph of the lobster *Homarus americanus*. J. Fish. Res. Bd. Canada 26: 1392–1397.

The levels of fluorescent material in the hemolymph of lobsters injected with serum proteins from lobster hemolymph labelled with fluorescein remained relatively constant over a 6-day test period; the levels in lobsters injected with bovine serum albumin labelled with fluorescein declined rapidly. A precipitin-like reaction was observed when lobster hemolymph serum was titrated with bovine serum albumin in vitro.

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FOREIGN PROTEIN has been shown to be eliminated readily from the circulatory system of a crustacean *Cambarus virilis* (Teague and Friou, 1964). The evidence indicated that the foreign protein was catabolized leading these authors to believe that the clearance was the result of a nonspecific degradation of the foreign material. Feng (1965) working with a mollusc, the oyster, has shown that another clearance mechanism can be operative in invertebrates, i.e., the foreign protein first concentrates in the hosts' leucocytes (pinocytosis) and is then eliminated from the animal by migration of the protein-laden cells to the exterior. Our work with the lobster *Homarus americanus* suggests that pinocytosis is not an important mechanism in this crustacean and that a precipitin-like principle already present in the hemolymph might be involved in the initial removal of foreign protein.

Fluorescein-labelled proteins were prepared by following the essential elements of the procedure of Rinderknecht (1962) for the preparation of immunofluorescent reagents. Leaching of the dye from the labelled proteins by the high salt concentration of lobster hemolymph was guarded against by using a medium isotonic with the hemolymph (artificial sea water (ASW); Stewart et al., 1967) to elute the labelled proteins from the Sephadex G 50 (Pharmacia, Uppsala, Sweden) columns prepared with ASW. The ASW was lightly buffered, 0.01 M Tris (tris (hydroxymethyl) amino methane), and maintained at *p*H 7.6, the normal *p*H of lobster hemolymph (Stewart et al., 1966). The protein preparations labelled were bovine serum albumin (BSA) (Pro-Sol, Standard Scientific Supply Corporation, New York 3, N.Y.) and lobster serum proteins (Stewart et al., 1966). Dialysis against ASW of labelled BSA and labelled hemolymph proteins eluted from the Sephadex column showed that the fluorescent label was stable in the ASW. Protein concentrations were determined by Robinson and Hogden's modification of the biuret method (Layne, 1957), and fluorescence was measured with a Turner Fluorometer (primary filter 47B-2A-12 and secondary filter 58-2A-12) or detected microscopically using a Spencer Fluorolume 645 ultraviolet source equipped with exciter filters A0693 and A0702 and barrier filter A0796 (American Optical Co., Instrument Division, Buffalo, N.Y.).

The lobsters used in this study were captured at Eastern Passage, N.S., and were kept in continuously flowing, aerated sea water at approximately 5 C. After being acclimated for a 2-week period, the lobsters were injected (in their ventral abdominal sinuses) with the labelled proteins. The level of fluorescence in the hemolymph was followed by removing, from the ventral abdominal sinus, 0.5 ml of hemolymph with a 1.0-ml syringe containing 0.5 ml of diluting fluid, (ASW at *p*H 7.6, 0.01 M Tris buffer, plus 0.05% Tween 80 (polyoxyethylene sorbitan monooleate) (Stewart et al., 1967)); this sample was then mixed immediately with a known volume of the diluting fluid, stirred for 1 min, and placed in a centrifuge for 15 min at 15,000 × *g*. The levels of fluorescence were determined on the supernatant fluids; a pre-injection sample (normal hemolymph) treated in the same manner as the post-injection sample served as a blank. Hemolymph smears for microscopic fluorescent examination were made in parallel with the quantitative measurements from hemolymph removed from the ventral abdominal sinus and the antennae. The results of the quantitative measurements are presented in Fig. 1 and 2.

Two hr after injection with the lobster serum proteins labelled with fluorescein, a plateau was reached that was maintained at the same order of magnitude for the term of the experiment (6 days) (Fig. 1) and was indicative of the unchanged nature of the proteins and the stability of the fluorescent label. In contrast, the labelled BSA never reached a state of equilibrium. The rate of clearance of labelled BSA was concentration-dependent (Fig. 2) unlike the phage clearance system reported for the spiny lobster by Cushing

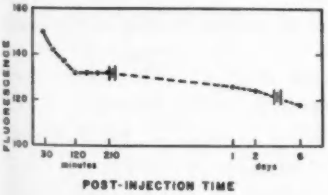

FIG. 1. Mean clearance rates for six lobsters injected with 1.0 ml of serum proteins from lobster hemolymph labelled with fluorescein. Temperature approximately 5 C; mean animal weight, 348 g. Fluorescence values recorded are: 1/40 dilutions of hemolymph on the 10× scale.

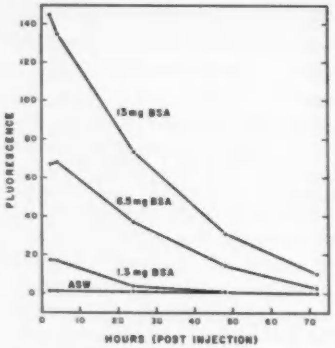

Fig. 2. Effect of concentration on rate of clearance of bovine serum albumin (BSA) labelled with fluorescein. Each curve represents the average values for three lobsters each injected with 1.0 ml of labelled BSA or 1.0 ml artificial seawater (ASW) as a control. Temperature 5 C; mean animal weight, 495 g. Fluorescence values recorded are 1/40 dilutions on 10× scale.

(1967). The appearance of fluorescent material in the water occupied by the injected lobsters was roughly proportional to the amount injected and coincided approximately with the disappearance of the fluorescent material from the hemolymph (Fig. 3). Surprisingly high levels of fluorescent materials were also excreted by the control lobsters (injected with ASW). The rate of clearance was temperature-dependent; the equivalent reduction of fluorescence levels in lobsters injected with equal doses of labelled BSA occurred twice as rapidly in lobsters kept at 15 C as it did in lobsters at 7 C.

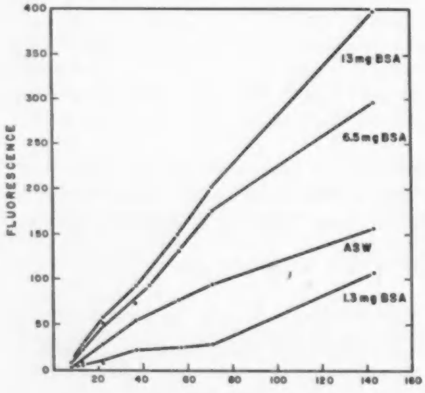

Fig. 3. Fluorescence levels observed in sea water occupied by lobsters cited in Fig. 2. Each group of animals was placed in 12 liters of water for the course of the experiment. Samples taken at intervals were centrifuged at 30,000 × *g* for 15 min at 4 C prior to determining fluorescence levels. Fluorescence values on 10× scale multiplied by factor of dilution required for reading on this scale.

The hemolymph smears (fixed with 0.4% iodine or 2% formaldehyde) made from lobsters injected with labelled BSA were examined in considerable detail by fluorescence microscopy. Fluorescent material was not detected in the intact hemocytes. Further trials using a large group of lobsters injected with high concentrations of a colored protein were run in an attempt to determine whether pinocytosis actually occurred but had gone undetected. Each lobster was injected with 10 ml of 2% unlabelled bovine hemoglobin in ASW; pairs of these animals were killed 15 min, 30 min, 1, 4, 8, 12, 24, 48, and 72 hr after the injection. The hearts were excised, and smears were prepared from the cardiac hemolymph and tissue and were examined microscopically. The hemoglobin could be observed in the hemolymph of the heart during the first ½-hr only. It did not appear to be precipitated in vivo in the heart and was not apparent in the hemocytes or cardiac tissue. The hemoglobin was not detected in any of the heart samples taken from lobsters killed subsequently. This is in contrast to the process of phagocytosis in the lobster; Cornick and Stewart (1968) showed that the cardiac hemocytes and cells within the cardiac tissue contained bacteria within 1 hr of the injection of suspensions of bacteria. Thus we have been unable to demonstrate pinocytosis in the American lobster.

Tiny granular accumulations of fluorescent material had been noted in the plasma surrounding the hemocytes only in lobsters injected with labelled BSA. These occurred approximately 8 hr after the injection when hemolymph fluorescence values were declining (Fig. 2). Attempts were made to determine whether the fluorescent proteins were concentrating in the hepatopancreas or green gland, but the natural fluorescence of these glands was too high and variable for this to be determined accurately.

The presence of tiny granules of fluorescent material in the hemolymph indicated that a precipitin-like principle might be operative. The standard ring test as outlined by Evans (1957) was used in an attempt to detect the precipitation of the foreign protein; freshly prepared ASW at *p*H 7.6 was used in place of physiological saline. These in vitro determinations showed that when labelled or unlabelled BSA (5 mg/ml in freshly prepared ASW) was placed in direct contact with lobster hemolymph serum a clearly discernable ring formed after 16 hr incubation at room temperature and a precipitate could be observed on the bottom of the tube after approximately 36 hr incubation. When labelled BSA was used as the immunogen the precipitate was fluorescent. The fluorescein-labelled BSA reacted with the lobster serum sooner, gave a higher titer, and a larger precipitate was formed than when unlabelled BSA was used. A 16-fold dilution of the serum was the maximum for which a positive reaction was observed; titers of 1:2 and 1:4 were more usual. The precipitin titer was independent of the protein concentration of the hemolymph serum. Dialysis of the lobster serum for approximately 8 hr against three changes of 1, 2, or 3% NaCl did not change the serum titer.

The stability of the precipitin to heat was tested by incubating the hemolymph serum for 5, 10, and 15 min at 30, 40, 50, 56, and 60 C, followed by centrifugation at 30,000 × *g* for 15 min at 4 C. The supernatant solution was tested for activity. The precipitin-like reaction was still active in all samples up to and including those held for 5 min at 50 C; longer times at 50 C and all times at higher temperatures destroyed the activity.

The relatively rapid disappearance of fluorescein-labelled BSA from the lobster's hemolymph contrasted markedly with what we assume is the relatively normal "biological decay" rate of the labelled homologous lobster hemolymph serum proteins. The clearance mechanism seems to discriminate readily between "self" and "not self" proteins and moreover appears to have an intrinsically high capacity as illustrated by the accelerated clearance of the higher BSA concentrations. The undiluted serum of many of the lobsters tested in vitro did give rise to large precipitates of foreign protein but the effect was lost rapidly upon dilution. It is possible that the principle responsible for clearance of foreign protein is maintained at relatively low levels in the circulatory system to be supplemented by further secretions upon demand rather than by de novo synthesis. Thus this would not necessarily entail a specific acquired immunological response.

An immunological response similar to that observed by Evans et al. (1968) after the injection of spiny lobsters with bacteria was not observed for the precipitin-like reaction of the American lobster. We injected 12 lobsters with 6.5 mg of labelled BSA and measured the precipitin levels at 0, 2, 5, 26, 48, and 72 hr post injection. The levels of precipitin did not increase but instead remained low or decreased. The response would be expected to occur within this time interval since the invertebrate responses to immunogens thus far have been shown to be immediate, short lived, and nonspecific (Chadwick, 1967; Evans et al., 1968).

Teague and Friou (1964) have suggested that a nonspecific (proteolytic) degradation might be involved in the clearance. Our data concerning the disappearance of the foreign protein and the excretion of fluorescent material is consistent with this. It is possible that the precipitin-like principle is the prime removal factor in a continuous removal with the digestion taking place elsewhere possibly in the hepatopancreas.

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REFERENCES

Chadwick, J. S. 1967. Serological responses of insects. Federation Proc. 26: 1675–1679.

Cornick, J. W., and J. E. Stewart. 1968. Interaction of the pathogen *Gaffkya homari* with natural defence mechanisms of *Homarus americanus*. J. Fish. Res. Bd. Canada 25: 695–709.

Cushing, J. E. 1967. Invertebrates, immunology and evolution. Federation Proc. 26: 1666–1670.

Evans, E. E. 1957. Serological methods, p. 211. *In* Manual of microbiological methods. Soc. Am. Bacteriologists, McGraw-Hill Book Co. Inc., New York.

Evans, E. E., B. Painter, M. L. Evans, P. Weinheimer, and R. T. Acton. 1968. An induced bactericidin in the spiny lobster, *Panulirus argus*. Proc. Soc. Exptl. Biol. Med. 128: 394–398.

Feng, S. Y. 1965. Pinocytosis of proteins by oyster leucocytes. Biol. Bull. 129: 95–105.

Layne, E. 1957. Spectrophotometric and turbidimetric methods for measuring proteins, p. 450. *In* S. P. Colowick and N. O. Kaplan [ed.] Methods in enzymology, Vol. 3. Academic Press, Inc., New York.

Rinderknecht, H. 1962. Ultra-rapid fluorescent labelling of proteins. Nature 193: 167–168.

Stewart, J. E., J. W. Cornick, and J. R. Dingle. 1967. An electronic method for counting lobster (*Homarus americanus* Milne Edwards) hemocytes and the influence of diet on hemocyte numbers and hemolymph proteins. Canadian J. Zool. 45: 291–304.

Stewart, J. E., J. R. Dingle, and P. H. Odense. 1966. Constituents of the hemolymph of the lobster *Homarus americanus* Milne Edwards. Canadian J. Biochem. 44: 1447–1459.

Teague, P. O., and G. J. Friou. 1964. Lack of immunological responses by an invertebrate. Comp. Biochem. Physiol. 12: 471–478.

Blood Protein Polymorphism in Harp Seals off Eastern Canada

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Nævdal, Gunnar. 1969. Blood protein polymorphism in harp seals off eastern Canada. J. Fish. Res. Bd. Canada 26: 1397–1399.

Blood samples from 208 harp seals taken in the Gulf of St. Lawrence and 175 taken off the east coast of Newfoundland showed no significant difference in frequency distributions of the serum transferrins or in gene frequencies. The samples were collected in the pupping season and analyzed by zone electrophoresis.

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THE PRESENT NOTE deals with comparisons of distributions of serum protein types in samples from harp seals, *Pagophilus groenlandicus* (Erxleben), breeding in the Gulf of St. Lawrence (the Gulf) and off the northeast coast of Newfoundland (the Front). Reasons for regarding the Front and Gulf herds as separate units were summarized by Sergeant (1965) but tagging experiments (Sergeant, MS, 1967, MS, 1968) indicate that the two herds mingle with each other to a certain extent.

In a previous paper (Nævdal, 1966) six separate types of serum transferrins were described from samples collected at all breeding and moulting areas of the harp seal except the Gulf of St. Lawrence. The six transferrin phenotypes were found to be controlled by three codominant autosomal alleles (Tf^{A}, Tf^{B}, and Tf^{C}), and significant differences at $p = 0.05$ (but not at $P = 0.02$) were observed in frequencies of two of the alleles between the sample from the Front and all samples from the northeast Atlantic.

Material and methods — Samples from the Front were collected during sealing seasons by observers from the Institute of Marine Research, Bergen, on board commercial sealing vessels in 1964 (Nævdal, 1966) and 1967. The sample from the Gulf was collected during a helicopter expedition organized by Arctic Biological Station, Fisheries Research Board of Canada, prior to the

TABLE 1. Distributions of transferrin groups and gene frequencies in harp seals in two areas. obs = observed distributions; exp = expected Hardy–Weinberg distributions.

Area and year of sampling	No.	Transferrin groups												Gene frequencies		
		Tf^{AA}		Tf^{AB}		Tf^{BB}		Tf^{AC}		Tf^{BC}		Tf^{CC}				
		obs	exp	obs	exp	obs	exp	obs	exp	obs	exp	obs	exp	q_A	q_B	q_C
Front 1964	78	3	5.9	31	27.0	29	30.8	6	4.1	9	9.4	–	0.7	0.28	0.63	0.10
Front 1967	97	13	13.4	37	37.5	26	26.3	9	7.8	12	10.9	–	1.1	0.37	0.52	0.11
Total Front	175	16	18.9	68	65.5	55	56.7	15	11.9	21	20.1	–	1.9	0.33	0.57	0.10
Gulf 1968	208	21	22.9	72	76.0	69	62.9	19	16.3	24	27.0	3	2.9	0.33	0.55	0.12

sealing season in 1968. Only specimens collected in breeding patches were used for these comparisons, and consequently they were adult females, or pups. In 1968 the blood was drawn from live seals by puncture of a vein in the hind flipper. Otherwise sampling, storage, and analyses by electrophoresis were made as formerly described (Nævdal, 1966). The number of specimens in each sample are shown in Table 1.

Results and discussion — All specimens showed the normal hemoglobin patterns described before (Nævdal, 1966), i.e., one major component (Hb A) moving towards the anode and one minor component (Hb A_2) moving towards the cathode at *p*H 9.0.

The Front sample for 1967 showed higher frequency of the gene Tf^A, q_A, and lower frequency of the gene Tf^B, q_B, than the sample for 1964. This observation implies that the statistical difference observed before, between samples from eastern and western populations, does not represent a real difference between the populations, because this difference was based on low values of q_A and high values of q_B in the 1964 Front sample.

No marked difference was found in the distributions of the transferrin groups or in the gene frequencies (Table 1). Therefore the present study has not revealed any clear difference between Front and Gulf harp seals, and on this basis it can not be decided whether the two herds are separated or not. However, even if isolation is not effective, it is still possible that the herds are isolated to such a degree that for practical management purposes they may be regarded as separate and self-sustaining units.

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REFERENCES

NÆVDAL, G. 1966. Protein polymorphism used for identification of harp seal populations. Årbok Univ. Bergen 1965 Mat.-Nat. Ser. 9: 1–20.

SERGEANT, D. E. 1965. Migration of harp seals *Pagophilus groenlandicus* (Erxleben) in the northwest Atlantic. J. Fish. Res. Bd. Canada 22: 433–464.

MS, 1967. Canadian research on harp seals in 1967, with further results from 1966 and previous years. Intern. Comm. Northwest Atlantic Fish. Ser. 1952: 1–22. (Mimeo.)

MS, 1968. Preliminary report on Canadian research on harp seals in 1968. Intern. Comm. Northwest Atlantic Fish. Ser. 2057: 1–4. (Mimeo.)

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Limited Fertilization of Steelhead Trout Eggs with Cryo-Preserved Sperm[1,2]

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GRAYBILL, J. R., AND H. F. HORTON. 1969. Limited fertilization of steelhead trout eggs with cryo-preserved sperm. J. Fish. Res. Bd. Canada 26: 1400–1404.

Results are reported on the fertilization (to 18%) of steelhead trout (*Salmo gairdneri*) eggs with cryo-preserved sperm. Fresh semen was progressively diluted to 1:4 with an extender containing 5 or 12.5% dimethyl sulfoxide, sealed in 1.0-ml ampoules, frozen in the vapor of liquid nitrogen, and subsequently thawed in a 4 C water bath. The fertilized eggs and alevins produced appeared normal when compared with those from fresh spermatozoa.

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FOR THE PAST CENTURY, scientists have experimented with methods to preserve viable fish sperm (de Quatrefages, 1853; Ellis and Jones, 1939; Blaxter, 1953; Sneed and Clemens, 1956; Mitchum, MS, 1963; Truscott et al., 1968). Except for Hoyle and Idler's (1968) work with Atlantic salmon (*Salmo salar*), attempts to fertilize the eggs of salmonids with cryo-preserved sperm have failed.

In this paper, data are presented on the successful fertilization (to 18%) of steelhead trout (*Salmo gairdneri*) eggs with sperm frozen 14 and 28 days at −196 C. The investigation was conducted during fiscal 1966–67, as part of a study to develop methodology for the cryo-preservation of viable salmonid sperm. Development of a practical sperm storage technique would facilitate more efficient fish cultural practices and enchance research into fish genetics, disease control, and manipulation of stocks.

One of the most successful experiments with cryo-preserved sperm of fish was conducted by Blaxter (1953.) He stored two sections of the testis of a March-spawning herring (*Clupea harengus*) in 80% sea water and 12.5% glycerol at −79 C. The stored sperm fertilized 80 and 85% of the eggs from autumn-spawning herring collected 6 months later. Sneed and Clemens (1956) froze the semen of carp (presumably *Cyprinus carpio*) in frog Ringer's solution containing 6–12% glycerol for 60 days and obtained 20% survival of the spermatozoa. Hodgins and Ridgway (1964) recovered motile and morphologically intact chinook salmon (*Oncorhynchus tshawytscha*) and pink salmon (*O. gorbuscha*) spermatozoa which had been frozen 7 days with LN_2 (liquid nitrogen) in a citrate-dextrose-sodium chloride solution containing dimethyl

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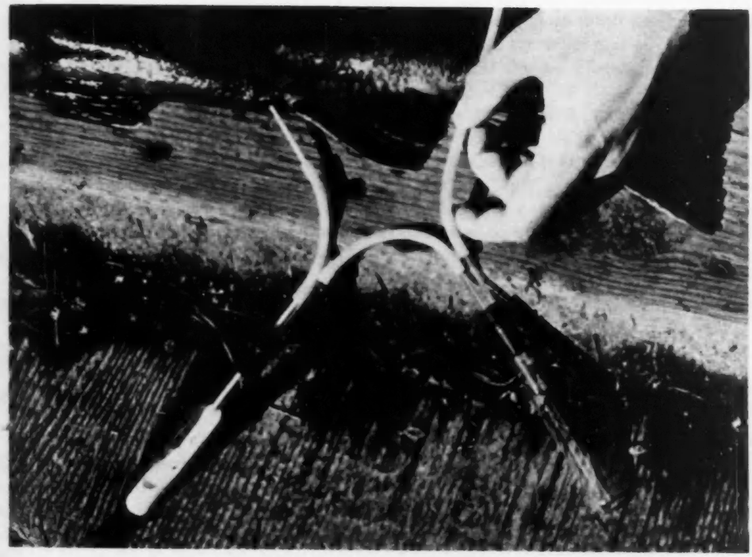

FIG. 1. The dual tube device used to collect samples of semen (the fish is positioned on its side for illustrative purposes).

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sulfoxide (DMSO). Fertilization (to 12%) of Atlantic salmon eggs with sperm frozen 30 min at –25 C was reported by Hoyle and Idler (1968). They used a modified Cortland medium containing 2.5% lactose with 5% ethylene glycol.

The development of techniques and procedures used in this paper is given in detail in our contract report to the U.S. Bureau of Commercial Fisheries (Horton et al., MS, 1967).

General methods — The apparatus used to collect semen was similar to that described by Miller (1965). Our device was made of two sterilized test tubes connected in series by rubber tubing. Small diameter glass tubes were inserted into the free ends of the rubber tubing. Mature steelhead trout from the Eagle Creek National Fish Hatchery were anesthetized in M.S. 222 (tricaine methanesulfonate), and held head up while one of the glass tubes was inserted into the genital pore. Oral suction to withdraw semen was applied at the other end of the tubing by the collector. The first test tube held the collected semen, and the second tube acted as a trap to collect saliva or excess semen (Fig. 1).

After the semen was collected, the test tubes were plugged and placed in a rack immersed in river water inside a styrofoam cooler. River water was used to avoid possible temperature shock to the spermatozoa. The temperature of semen transported thus to our laboratory increased a maximum of 3 degrees C on a warm day and a 3-hr trip.

At the laboratory, the styrofoam cooler was placed in a walk-in refrigerator maintained at 4 C. When the water temperature in the cooler decreased to 4 C, the rack of tubes was removed. The semen was mixed 1:2 with Extender 48, the chemical constituents of which were:

Component:	NaCl	KCl	$CaCl_2 \cdot 2H_2O$	$NaHCO_3$	NaH_2PO_4	$MgSO_4 \cdot 7H_2O$	Fructose	Lecithin (soy bean, crude)[3]
Molarity	0.125	0.00509	0.00157	0.00119	0.00297	0.000933	0.00555	
g/liter								5.00

After 30 min the extended semen was mixed 3:2 with Extender 48 to which 10 or 25% DMSO had been added. Thus, the final dilution of semen was 1:4 with Extender 48 of which 5 or 12.5% was DMSO. The extender[4] and protector[5] (DMSO) were equilibrated to 4 C. Sperm from different males was never mixed.

Extended and protected semen was placed into 1.0-ml glass ampoules, which were sealed by heat[6] inside the walk-in refrigerator. Heat from the torch or the molten glass had no detectable effect on the sperm. One ampoule was left unsealed to receive a thermocouple used to give continuous temperature read-out during the freezing process.

Ampoules of semen were frozen by either slow or rapid methods. If a slow rate of temperature decrease (1–5 degrees C/min) was desired, the ampoules were frozen in a LN_2 freezer chamber.[7] Continuous temperature read-out was recorded on a strip-chart recorder.[8] The temperature of the freezing unit was lowered by a controller[9] at a predetermined rate until the semen reached −120 C. Ampoules were then removed from the freezing chamber, placed on canes, and stored in a LN_2 refrigerator [10] at −170 to −196 C. All samples frozen at any one time were of the same characteristics with regard to volume, amount of extender, and amount of protector.

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[3] Nutritional Biochemical Corporation, 21010 Miles Avenue, Cleveland, Ohio 44128.

[4] Extender (life extender or diluent): a solution of salts, sometimes including organic compounds, which helps maintain the viability of cells during refrigeration.

[5] Protector (life protector or additive): an organic compound which protects the viability of cells during the freezing and thawing processes.

[6] Ampoule Sealer, Model 161, Kahlenberg-Globe Equipment Co., Box 2803, Sarasota, Florida.

[7] Freezing Chamber, Model BF-4B, Union Carbide Corp., Linde Division, Cryogenic Products Dept., 2770 Leonis Blvd., Los Angeles, Calif. 90058.

[8] Strip-chart Recorder, Model G-11A, Varian Associates, 611 Hansen Way, Palo Alto, Calif.

If a rapid temperature decrease of around 30 degrees C/min was desired, the ampoules were suspended in vapor inside the LN_2 refrigerator.

Ampoules of frozen semen were thawed by immersing them in a 4 C water bath.

Experiments — The capacity of cryo-preserved sperm to fertilize eggs was tested comparatively with fresh sperm. Fresh semen from a steelhead trout was collected and examined for motility. Ampoules of semen frozen 2 weeks previously from two steelhead trout were selected and examined for motility. One ampoule from each fish had been frozen in Extender 48, of which 5% was DMSO, at a temperature decrease of 1 degree C/min to −30C, and then 5 degrees C/min until the end of the freezing process. A second ampoule from each fish had been mixed in the same extender and protector, but was frozen in LN_2 vapor.

About 200 eggs from a steelhead trout were placed into each of three, 4-oz jars. Fresh semen, 0.2 ml, was placed into one jar and 1.0 ml of thawed semen (frozen in nitrogen vapor) from each of the two fish was placed into one or the other of the two remaining jars. About 200 eggs from another steelhead trout were placed into each of three more jars. In this test, 0.2 ml of fresh semen was placed into one jar and 1.0 ml of thawed semen (frozen initially at 1 degree C/min) from each of the two fish was added to one or the other of the two remaining jars. Eggs and sperm were gently mixed. Excess sperm was washed away and the eggs were placed in incubators and bathed with separate water supplies. The time elapsed between thawing the first sample and mixing it with eggs and thawing the fourth sample and mixing it with eggs ranged from about 2–5 min respectively.

Two weeks later, a similar experiment was performed. About 200 eggs from a female were stripped into each of four jars. Then 1 ml of thawed semen (frozen in LN_2 vapor) from each of two fish was introduced into separate jars of eggs. Semen was not added to the third jar, which served as a control to reveal possible contamination by other sperm or to show parthenogenic development. Fresh semen, 0.2 ml, was added to the fourth jar. Excess sperm was washed away by running stream water from a hose over the eggs contained in each jar.

This procedure was repeated for semen frozen initially at a temperature decrease of 1 degree C/min and for semen frozen in LN_2 vapor, except that in the latter test, semen in one of the ampoules had been protected with 12.5% DMSO.

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[9] Controller, Model BF-4, Union Carbide Corp., Linde Division, Cryogenic Products Dept., 2770 Leonis Blvd., Los Angeles, Calif. 90058.

[10] Biostat, Model 1000-X, Cryogenic Engineering Co., 4955 Bannock St., Denver, Colorado 80216.

Except for controls, only those eggs exposed to sperm frozen in LN_2 vapor were fertilized (Table 1).

After 36–39 degree-days above freezing, a sample of six eggs was removed from each lot of the second experiment and preserved in Carnoy's fixative (1 part glacial acetic acid to 3 parts 95% ethanol). These eggs were checked for fertilization and two from each group were examined for numbers and morphology of chromosomes.

TABLE 1. Per cent fertilization of eggs with steelhead trout semen frozen April 14, 1967, at two rates of temperature decrease in Extender 48 of which 5 or 12.5% was DMSO, sperm having been mixed with eggs on one or other of two dates.

Female no.	Male no.	% of DMSO	Freeze rate	% fertilized
		Eggs and sperm mixed Apr. 28, 1967[a]		
1	110-Control	–	Fresh, not frozen	87.2
	95	5	LN_2 vapor	18.0
	101	5	LN_2 vapor	3.3
2	110-Control	–	Fresh, not frozen	95.0
	95	5	1–5 C/min	0.0
	101	5	1–5 C/min	0.0
		Eggs and sperm mixed May 12, 1967[b]		
3	115-Control	–	Fresh, not frozen	98.3
	95	5	LN_2 vapor	6.1
	101	5	LN_2 vapor	0.0
	Blank	–	–	0.0
4	115-Control	–	Fresh, not frozen	97.6
	95	5	1–5 C/min	0.0
	101	5	1–5 C/min	0.0
	Blank	–	–	0.0
5	115-Control	–	Fresh, not frozen	99.3
	106	5	LN_2 vapor	0.5
	106	12.5	LN_2 vapor	0.8
	Blank	–	–	0.0

[a] Per cent fertilization was computed from counts of alevins and dead eggs.
[b] Per cent fertilization was computed from counts of eyed and dead eggs.

Our confidence in the chromosome analysis was reduced because of the low success of fertilization. Blank cells were found in all eggs sampled except controls and one egg from female 3 fertilized with sperm from male 95. Based on cell numbers and size, the rate of development for the egg fertilized with cryo-preserved sperm was the same as for the controls. No chromatin bridges nor lagging chromosomes were found in cells in anaphase, and no haploid cells were found. From all indications, the blastula was normal.

Discussion — A possible reason that only sperm frozen in nitrogen vapor fertilized eggs is because the cells were rapidly cooled so as to bypass the zone of crystallization and assume the vitreous state. Rapid warming of the frozen cells in a water bath as opposed to air may also help avoid the zone of crystallization. Bypassing the zone of crystallization is reported to prevent changes such as denaturation and coagulation of proteins, protoplasmic precipitation, release of enzymes, and structural disarrangement (Mann, 1964).

Because only sperm frozen in LN_2 vapor fertilized eggs, and because no special chamber, control, or electricity is required, freezing sperm in LN_2 vapor could be a practical field technique. Portable freezing equipment could be used at isolated field stations to preserve quantities of semen for future use.

Truscott et al. (1968) emphasized that the true test of viability of sperm is the capacity to fertilize. Our test of fertilizing capacity was designed to show only if sperm which had been frozen could fertilize fresh eggs. Because of the particular test used, we cannot say whether per cent fertilization was related to the way semen was frozen, or to the time elapsed between thawing and mixing the sperm with the eggs. Because these results were not obtained until after the end of the spawning season, further tests were not possible.

To our knowledge, this was the first successful attempt to fertilize the eggs of a salmonid with spermatozoa which had been frozen. Alevins 7 days old produced from eggs fertilized with frozen sperm appeared normal when compared with those produced from fresh spermatozoa.

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REFERENCES

Blaxter, J. H. S. 1953. Sperm storage and cross-fertilization of spring and autumn spawning herring. Nature 172: 1189–1190.

Ellis, W. G., and J. W. Jones. 1939. The activity of the spermatozoa of *Salmo salar* in relation to osmotic pressure. J. Exptl. Biol. 16: 530–534.

Hodgins, H. O., and G. J. Ridgway. 1964. Recovery of viable salmon spermatozoa after fast-freezing. Progressive Fish-Culturist 26: 95.

Horton, H. F., J. R. Graybill, and A. S. H. Wu. MS, 1967. Cryogenic preservation of viable fish sperm. Contract Completion Rept., Dept. Fish. Wildlife, Oregon State Univ., Corvallis, Ore. 93 p.

Hoyle, R. J., and D. R. Idler. 1968. Preliminary results in the fertilization of eggs with frozen sperm of Atlantic salmon (*Salmo salar*). J. Fish. Res. Bd. Canada 25: 1295–1297.

Mann, T. 1964. The biochemistry of semen and of the male reproductive tract. John Wiley, New York. 493 p.

Miller, J. G. 1965. Advances in the use of air in taking eggs from trout. Progressive Fish-Culturist 27: 234–237.

Mitchum, D. H. MS, 1963. Study of the preservation of fish sperm by freezing. Wyoming Game Fish. Comm. Completion Rept. FW-3-R-10: 33–37.

Quatrefages, A. de. 1853. Recherches sur la vitalité des spermatozoides de quelques poissons d'eau douce. Ann. Sci. Naturalist 19: 341–369.

Sneed, K. E., and H. P. Clemens. 1956. Survival of fish sperm after freezing and storage at low temperatures. Progressive Fish-Culturist 18: 99–103.

Truscott, B., D. R. Idler, R. J. Hoyle, and H. C. Freeman. 1968. Sub-zero preservation of Atlantic salmon sperm. J. Fish. Res. Bd. Canada 25: 363–372.

Recent Articles on FRB Investigations Published in Other Periodicals

Studies No.

1291. Sprague, John B. 1968. Promising anti-pollutant: chelating agent NTA protects fish from copper and zinc. Nature 220: 1345–1346.

1292. Yamazaki, Fumio, and Edward M. Donaldson. 1968. The effects of partially purified salmon pituitary gonadotropin on spermatogenesis, vitellogenesis, and ovulation in hypophysectomized goldfish (*Carassius auratus*). Gen. Comp. Endocrinol. 11: 292–299.

1293. Parker, Robert R. 1968. *Caligus appendiculatus* F. Müller, 1852 (Crustacea: Copepoda): A request for suppression under the plenary powers. Z.N.(S.) 1839. Bull. Zool. Nomenclature 25: 105–106.

1294. Parsons, T. R., and D. J. Blackbourn. 1968. Pigments of the ciliate *Mesodinium rubrum* (Lohmann). Neth. J. Sea Res. 4: 27–31.

1295. Parsons, T. R., and R. J. LeBrasseur. 1968. A discussion of some critical indices of primary and secondary production for largescale ocean surveys. Calif. Marine Res. Comm. CalCOFI Rept. 12: 54–63.

1296. Jordan, Fred P., and Howard D. Smith. 1968. An aluminum staple tag for population estimates of salmon smolts. Progressive Fish-Culturist 30: 230–234.

1297. Bell, Gordon R., Gary E. Hoskins, and John W. Bagshaw. 1969. On the structure and enzymatic degradation of the external membrane of the salmon egg. Canadian J. Zool 47: 146–148.

1298. Margolis, L., and Z. Kabata. 1967. The structure of the buccal region of *Salvelinema* Trofimenko, 1962 (Nematoda: Cystidicolinae). Canadian J. Zool. 45: 1067–1072.

1299. Margolis, L. 1967. The swimbladder nematodes (Cystidicolinae) of Pacific salmons (genus *Oncorhynchus*). Canadian J. Zool. 45: 1183–1199.

1300. Margolis, L. 1967. Blood feeding in *Salvelinema walkeri* (Nematoda: Cystidicolinae), a parasite of coho salmon (*Oncorhynchus kisutch*). Canadian J. Zool. 45: 1295–1296.

1301. Kabata, Z. 1968. *Praecidochondria galatheae* gen. et sp.nov. (Copepoda, Chondracanthidae) from a Malayan fish. Vidensk. Medd. Dansk. Naturhist. Foren. 131: 99–103.

1302. Shepard, M. P., A. C. Hartt, and T. Yonemori. 1968. Salmon of the North Pacific Ocean. Part VIII. Chum salmon in offshore waters. Intern. North Pacific Fish. Comm. Bull. 25: 69 p.

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